- 1 -

A METHOD OF INSPECTING A DNA CHIP AND

AN APPARATUS THEREOF

BACKGROUND OF THE INVENTION

The present invention relates to an apparatus for reading and analyzing an emitted light (fluorescent light) pattern emitted from a sample located on a
5   plane. More particularly, it relates to a method and an apparatus by which living body samples, such as a DNA, a RNA and an oligonucleotide marked by a fluorescent material, are trapped at a plurality of positions on a substrate so as to read an emitted light
10   pattern from the fluorescent marker with a high-resolution, at a high-speed and with a high-sensitivity.

Analysis technologies for analyzing the DNA and proteins are important in fields of medicine and
15   biology including gene analysis and gene diagnosis. In particular, in recent years, attention has been focused on a method and an apparatus by which, using a DNA probe array (which is called in other way by various names such as oligochip, DNA chip or biochip, but here-
20   inafter the DNA probe array is employed as the generic name), a variety types of DNA sequence information and gene information are inspected and analyzed simultaneously from one specimen to be inspected. The DNA probe array is implemented in the following way: A substrate

such as a glass is used and separated into a plurality of (millions to tens of millions) areas, and target (usually, different types of) DNA probes are fixed onto the respective areas so as to form the respective areas

5 into microscopic reaction areas, thus implementing the array. The reaction of the specimen with the array allows a target DNA in the specimen to be trapped in a state of being hybridized with the fixed DNA probes. Moreover, the target DNA is coupled with a probe such

10 as a fluorescent probe, thereby making it possible to measure the coupled state (the position, i.e., the hybridized sequence) and its quantity with the use of the fluorescence intensity and so on. This permits the array to be utilized in the gene diagnosis and the

15 sequence.

A microscope-like apparatus (confocal fluo-rescent microscope), which is usually called a scanner, is used in order to read the fluorescence intensity emitted from the fluorescent marker on the target DNA

20 trapped in the respective reaction areas of the DNA probe array (for example, refer to JP-A-11-315095). In this apparatus, the array is irradiated with a excita-tion light such as a laser light which has been modified into one microscopic spot-shaped configura-

25 tion, and fluorescent lights generated are separated from the excitation light using a spectroscopic device such as an interference filter, then detecting the fluorescence intensity using a photo detector such as a

photomultiplier tube. At that time, the excitation light is waved using a galvanometer mirror so as to scan, in a 2-dimensional manner, the microscopic spot formed on the array. Otherwise, the position of the

5   microscopic spot is fixed, then scanning the array in the 2-dimensional manner. These scans make it possible to recognize the fluorescence intensity distribution of the entire array, i.e., the degree of the coupling toward each DNA probe.

10   When trying to apply the DNA inspection to a living body inspection such as a conventional blood inspection, it becomes absolutely required to execute the inspection toward a large number of living specimens at a high-speed. In the conventional

15 technologies, however, it takes so much inspection time to irradiate one point with the excitation light spot, which is equivalent to a necessary inspecting resolu- tion of the DNA chip, and to detect the obtained fluorescent lights one after another. This is

20 concerned with the fact that it is impossible to shorten unlimitedly a time needed to detect this one point. Namely, this is attributed to the fact that the time Δt_L, which is needed up to the finishing of the fluorescent light generation after the irradiation with

25 the excitation light, is equal to substantially 10 ns. Transferring the detection to the next detection point without waiting for the finishing of the fluorescent light generation makes the detection itself impossible.

Also, it is required to perform the detection with a high-sensitivity that corresponds up to a state where there exist several fluorescent molecules within the spot light size equivalent to the above-described

5 necessary detecting resolution. However, all of the generated fluorescent lights are not detected. Namely, a light utilization efficiency of the detecting optical system and a quantum efficiency of the photomultiplier tube used for the optical detection are not equal to

10 100%. Moreover, the following values are small: An efficiency with which the excitation light is absorbed in the fluorescent material and a probability with which the absorbed excitation light is converted into the fluorescent light. This requires that the detec-

15 tion be executed by spending a time that is at least tens to hundreds times longer than Δt_L. Furthermore, the more this time is lengthened, the higher the accuracy of a weak light detection becomes which is almost the same as the photon counting.

20 Also, in order to accomplish such a high-speed characteristic at a practical level, the following conditions are required: Eliminating or reducing influences of foreign substances that are composed of various types of proteins mixed into the DNA chip, or

25 maintaining all the time a focal point of the detecting system onto the inspection plane on which the fluorescent material-added target has been hybridized. Also, in some cases, it becomes necessary to perform

the detection with reference to a plurality of fluorescent lights at a high-speed.

When forming multi-spot lights from a bean emitted from a laser light-source, if the emitted light
5 is wished to be modified into the multi-spot lights without being wasted, i.e., for example, when trying to form 50 multi-spot lights that are 0.04 mm in diameter and are arranged in a line with a pitch of 0.4 mm, the emitted laser beam is formed so that the beam becomes
10 an ellipse-shaped configuration the longitudinal-to-transverse ratio of which is equal to 1 to 50. Irradiating, with the ellipse-shaped beam, a microlens array where microlenses are arranged with a pitch of 0.4 mm makes it possible to obtain the 50 multi-spot
15 lights about 0.04 mm in diameter and having the pitch of 0.4 mm, if a focal length of the microlenses is equal to 60 mm, for example. Since the intensity distribution of the laser beam is Gaussian distribution, in the arrangement of the 50 multi-spot lights
20 formed in the method as described above, spots situated in proximity to the center of the arrangement become lighter and spots situated in proximity to the periphery thereof become darker. Trying to equate as much as possible the intensities of the spots at the
25 center with those of the spots on the periphery requires the following: A spread area of the incoming ellipse-shaped beam should be made large enough as compared with the width of the 50 microlenses. As a

result, half or more of the laser beam energy emitted from the laser light-source turns out to be wasted.

In the DNA probe array, the number of the types of the fixed probes is increased and thus its
5 density is becoming more and more heightened. On account of this, an apparatus for measuring the fluorescent marker distribution of the DNA probe array is expected to operate at the high-speed and with the high-sensitivity in addition to implementing the high-
10 resolution characteristic. In the above-described apparatus, however, no sufficient consideration has been given to this point. Namely, it is required to make the spot diameter of the excitation light more microscopic in order to implement the high-resolution
15 characteristic, whereas the scanning time is increased at that case (when an exposure time for each spot is identical). Moreover, making the spot diameter smaller lessens the number of the fluorescent molecules existing within the spot diameter, thereby lowering the
20 detection sensitivity. Also, in order to implement the high-speed characteristic, it is required to increase the scanning rate or to make larger the spot diameter of the excitation light. Increasing the scanning rate, however, shortens the excitation time for the fluo-
25 rescent material, thereby lessening the fluorescence intensity or increasing a circuit noise to lower the detection sensitivity. Also, in order to implement the high-sensitivity characteristic, it is required to make

the spot diameter larger and to delay the scanning rate. Namely, implementing the high-resolution characteristic, implementing the high-speed characteristic and implementing the high-sensitivity character-

5 istic are contradicted to each other, and accordingly it was difficult to accomplish them.

Also, there has been proposed an apparatus by which a plurality of microscopic spots are formed on a sample surface and lights from the plurality of

10 microscopic spots are detected simultaneously (for example, refer to JP-A-11-118446). In this configuration, a light-flux from an excitation light-source is enlarged and a 2-dimensional microlens array is located in the enlarged light-flux so as to form the plurality

15 of irradiation spots, then projecting the spots onto the sample surface. In this case, however, especially when a laser light is used as the excitation light, because of the Gaussian distribution characteristic of the laser beam, the light intensities of spots in

20 proximity to the center are intense and the light intensities of the spots become lower as their positions come nearer to the periphery. Accordingly, it is difficult to equate all the light intensities of the plurality of irradiation spots. This problem is

25 partly solved by enlarging the excitation light up to a light-flux the diameter of which is large enough as compared with the width of the 2-dimensional microlens array. In this case, however, the light utilization

efficiency is no good and the light intensities are decreased, thus resulting in such a problem that the detection sensitivity is deteriorated.

In a method of focusing a one-beam laser

5 light onto a target to be inspected, the detection is executed on a one-pixel by one-pixel basis in a time-sequence manner. As a result, when the fluorescent light is extremely weak, it takes a time to execute the detection equivalent to the one-pixel. Consequently,

10 it takes a considerable amount of time to detect the entire 2-dimensional image. Even if an excitation light of the one-beam laser is strengthened in order to detect the weak fluorescent light emitting target at a high-speed, when trying to detect, for example, 6000 ×

15 6000 pixels in 1 minute, the detecting time per pixel turns out to become 1 to 2 μsec (microseconds). Executing the detection in such a short time makes it difficult to detect the fluorescent light detection intensity in a broad dynamic range (for example, 2^{16}).

20 Also, since a normal fluorescent material emits the fluorescent light with a delay of 10^{-9} to 10^{-5} sec after receiving the excitation light, the above-described per-pixel fluorescent light detecting time of 1 to 2 μsec prevents the fluorescent light detection from

25 being fully accomplished. Meanwhile, in a method using Nippou disk, the light is utilized effectively only by the amount of a ratio of a microscopic aperture in a uniform light. This condition requires a considerable

amount of time to be spent to detect the weak fluo-
rescent light.

SUMMARY OF THE INVENTION

In order to solve the above-described

5   problems, the present invention provides methods and

units described hereinafter.

The inspection of a DNA chip to be inspected

is executed in the following steps: Irradiating

mutually different positions with a plurality of M

10   excitation lights simultaneously with the use of an

objective lens for a time Δt that is longer than a

fluorescent light attenuation time, the excitation

lights including a spot diameter d that is smaller than

a dimension D of each cell, guiding obtained fluo-

15   rescent lights to a fluorescent light detecting optical

path, detecting the fluorescent lights on an image-

forming plane that is in a conjugation relationship

with irradiation spots onto the DNA chip, and executing

the inspection of the DNA chip from positions and

20   intensities of the fluorescent lights. At this time,

the spot diameter is set to be substantially 1/N of an

integer N indicating the cell dimension. Moreover,

relative positions between the irradiation spots and

the DNA chip are caused to differ j times in sequence

25   so as to inspect the entire inspection target positions

$LN^2 = Mj$. Here, L denotes the cell number within the

chip. Also, N is made equal to 2 or more and each cell

is divided into a plurality of portions so as to select and process only significant data out of N^2 data existing within one call, thereby executing the precise inspection. Executing the inspection in this way

5 allows the fluorescent lights to be detected in a time less than $LN^2/(6 \times 10^5)$ sec with reference to the entire inspection target sample-point number LN^2 of the DNA chip.

There are provided M light-receiving aper-
10 tures that have an effective diameter on the image-forming plane that is in the conjugation relationship with the plurality of M irradiation spots onto the DNA chip, the effective diameter being of substantially the same order as an effective diameter of the irradiation
15 spot images. Moreover, an area other than the light-receiving apertures is light-shielded so as to detect the respective lights that have passed through the light-receiving apertures, thereby eliminating noise lights from the irradiation spots or from an area other
20 than the irradiation spot plane so as to perform the inspection having a high signal-to-noise ratio. Also, the M light-receiving apertures that have the effective diameter are optical fiber light-receiving ends, the effective diameter being of substantially the same
25 order as the effective diameter of the irradiation spot images. At the optical fiber light-receiving ends, the lights emitted from optical fiber light-emitting ends are detected, thereby performing the inspection having

an even higher signal-to-noise ratio.

The plurality of M irradiation spots onto the DNA chip are arranged on a straight line with a spacing of substantially kd with reference to the spot diameter

5   d and an integer k. After the irradiation with this spot array during the time Δt has been performed, the spot array is displaced in an array direction by a distance of substantially d, and then the irradiation during the time Δt is performed. This operation is

10   repeated k times in sequence, thereby performing the inspection concerning kM spot positions in the array direction. At the same time, the DNA chip and an inspecting apparatus are relatively displaced at least in a direction perpendicular to the array direction,

15   thereby inspecting a desired 2-dimensional area of the DNA chip. Also, the above-described movement of the spot array is characterized by being performed using an acoustooptic deflector.

Also, it is desirable to set the integer k to

20   be 2 or more. Furthermore, in view of the signal-to-noise ratio, it is more advantageous to set the integer k to be 5 or more.

The spot array is formed with the microlens array. The spot array can also be formed using a

25   hologram. In synchronization with the above-described movement of the spot array in the array direction, a fluorescent light detection deflecting unit is provided within the fluorescent light detecting optical path so

that the fluorescent lights generated by the excitation lights come onto substantially the same location on the above-described light-receiving apertures. At this time, a deflecting unit using a piezoelectric device is

5 employed as the fluorescent light detection deflecting unit. Also, the fluorescent light detection deflecting unit is configured with a wavelength selection beam splitter for permitting the excitation lights to pass through and reflecting the fluorescent lights. This

10 makes it possible to perform the detection with a higher efficiency. Also, in order to enhance the separation from the excitation lights, a filter for permitting only the fluorescent lights to pass through and light-shielding the excitation lights is used

15 within the fluorescent light detecting optical path isolated from excitation optical paths.

The above-described M multi excitation spot lights are configured so as to pass through substantially the same position A on a pupil of the objective

20 lens. In addition, the excitation lights reflected regularly on the DNA chip are configured so as to reach a position B' on the pupil of the objective lens. In this way, a light-shielding unit for light-shielding the reflected excitation lights is provided at the

25 position B' on the pupil of the objective lens, or is provided at an image position of B' that exists within the fluorescent light detecting optical path and exists on a plane conjugated with the pupil of the objective

lens. This allows the excitation lights becoming the noise components to be eliminated from a fluorescent light detection signal.

The above-described M multi excitation spot
5   lights are configured so as to pass through substantially the same position A on the pupil of the objective lens. In addition, the excitation lights reflected regularly on the DNA chip are configured so as to pass through a position B that differs from the
10 position A on the pupil of the objective lens. In this way, a light-shielding member for light-shielding the reflected excitation lights having a desired diameter with B as its center is located on the pupil of the objective lens, or is located at a position that is
15 within the fluorescent light detecting optical path and is conjugated with the pupil of the objective lens. This configuration allows the excitation lights becoming the noise components to be eliminated from the fluorescent light detection signal.
20 Moreover, the reflected excitation lights to be light-shielded are employed as the regularly reflected excitation lights. The excitation lights scattered from the foreign substances within the DNA chip are extracted by being caused to pass through from
25 outside the above-described light-shielding unit or the above-described light-shielding member. Then, the extracted scattered lights are branched off from the above-described fluorescent light detecting optical

path so as to image-pickup the scattered lights at a position conjugated with the irradiation spots onto the DNA chip, thereby performing the detection. Next, using the image-pickup information on images of the

5 detected scattered lights, the fluorescent light information detected by the above-described fluorescent light detection deflecting unit is corrected. This method precludes the influences of the scattered lights scattered from the foreign substances existing in the

10 DNA chip, thereby making it possible to execute the precise detection.

The above-described M multi excitation spot lights are formed using a laser light-source. This allows high-intensity excitation irradiation to be

15 accomplished on the microscopic spots. Also, the M multi-spot excitation lights are formed using a plurality of semiconductor laser light-sources, thereby accomplishing even higher excitation irradiation in a small implementation volume. At this time, the lights

20 emitted from the plurality of semiconductor laser light-sources are configured so that the lights are guided into optical fibers and are then emitted from the optical fiber light-emitting ends that are aligned with M desired pitches. This configuration makes it

25 possible to obtain the M multi excitation spot lights arranged in the desired pitch arrangement.

Employing, as an optical signal storage type image-pickup unit, a 2-dimensional image-pickup

apparatus having an ultra-high sensitivity and include-ing $N_x \times N_y$ pixels, and assuming that n_x, n_y are integers, $N_x \times N_y$ spots are simultaneously irradiated with the excitation lights of the spot diameter d with

5 a pitch of $n_x d$ in the x direction and with a pitch of $n_y d$ in the y direction. Then, $N_x \times N_y$ fluorescent light spot images obtained in this way are detected using the ultra-high sensitivity 2-dimensional image-pickup apparatus, and a relative position between the detect-

10 ing apparatus and the DNA chip is displaced with a pitch of d in the x, y directions by the amount of $n_x \times n_y$ steps. This method makes it possible to detect a desired area on the DNA chip.

Also, the above-described excitation lights,
15 which include a plurality of different wavelengths, separate and detect a plurality of fluorescent materials-added different targets. Furthermore, the plurality of fluorescent materials-added different targets are simultaneously irradiated with the excita-
20 tion lights including the plurality of different wavelengths, thereby separating and simultaneously detecting the different targets. This method makes it possible to detect various types of targets at high-speed.

25 On the inspection plane with which a desired fluorescent material-added target on the above-described DNA chip to be inspected has been hybridized, a second light is obliquely incoming into a proximity

to the above-described excitation spot lights and a position at which the second light is reflected on the inspection plane is detected, thereby detecting the focal point. A relative distance between the inspec-

5 tion plane and the above-described objective lens is controlled in accordance with this focal point information, thereby allowing the focusing to be achieved. Also, the obliquely launched second light is configured so as to pass through the objective lens. This permits

10 the focal point detection and control to be executed with a simplified configuration.

The above-described obliquely launched second light is converted into an S-polarized light toward the detection plane. This enhances a reflectance on the

15 fluorescent light detection plane, thereby making it possible to execute the correct focal point detection. In addition, as the wavelength of the obliquely launched second light, there is employed a wavelength that exerts no excitation to the above-described

20 fluorescent material. This permits the precise detection to be executed without superimposing the noise on the fluorescent light detection signal.

It is an object of the present invention to provide a method and an apparatus therefor that obtain

25 multi-spot lights having a substantially uniform intensity by solving a conventional problem that the light utilization efficiency in the conventional multi-spot light forming method is no good and there exist a

large variation in the intensities among the multi-spot
lights.

It is another object of the present invention
to provide a method and an apparatus therefor that
5   execute the confocal point detection, the fluorescent
light detection and the DNA inspection with the use of
the multi-spot lights having the substantially uniform
intensity.

In order to solve the conventional problem
10 and obtain multi-spot lights positioned in proximity to
each other, the present invention presents the follow-
ing configuration:

Namely, a plurality of N laser beams which
travel in substantially the same direction and the
15 adjacent beams of which are in comparative proximity to
each other are launched into a polarization component
so as to separate each laser beam into two beams of
mutually orthogonal polarized components, thereby
forming a plurality of 2N laser beams. After that, the
20 plurality of 2N laser beams are formed into a plurality
of 2N laser beams which travel in substantially the
same direction and the adjacent beams of which are in
comparative proximity to each other.

As a concrete method of obtaining such
25 doubled proximate beams, the plurality of N proximate
laser beams are launched into the above-described
polarization component including a multi-spot lights-
doubling prism that has a polarization beam split plane

and total reflection planes. Here, the total reflec-
tion planes are parallel to the polarization beam split
plane and are positioned at distances of L1 and L2 from
the polarization beam split plane. This makes it

5   possible to increase the number of the plurality of
proximate laser beams up to the doubled number, i.e.,
2N.

Also, the plurality of N proximate laser
beams are launched into the above-described polariza-

10 tion component including an anisotropic optical medium.
Then, in the anisotropic optical medium, each laser
beam is separated into the two beams of the mutually
orthogonal polarized components, thereby forming the
plurality of 2N laser beams. This makes it possible to

15 form the plurality of N proximate laser beams into the
plurality of 2N laser beams which travel in substan-
tially the same direction and the adjacent beams of
which are in comparative proximity to each other.

In addition, the use of the above-described

20 beam-doubling type polarization components in plural
number M makes it possible to obtain $2^M N$ laser beams.

The plurality of N proximate laser beams,
which are launched into at least one of the above-
described one or more of polarization components, are

25 formed into converging laser beams, respectively. This
allows the microscopic multi-spot lights to be obtained
at a convergence position of the converging laser
beams. At this time, the converging laser beams are

formed using lenses arranged in plural number, thereby forming the multi-spot lights.

Also, a mask, in which $2^{M'}N$ or more of apertures having a diameter substantially equal to a

5 convergence diameter of the multi-spot lights are arranged with reference to an integer M' satisfying $1 \leqq M' \leqq M$, is located at the convergence position of the above-described converging laser beams so that the multi-spot lights are focused onto the apertures. This

10 makes it possible to obtain pure microscopic multi-spot lights without the noise lights or stray lights.

The above-described plurality of N laser beams can also be doubled even when the plurality of N laser beams have a 4d or less of adjacent spacing pitch

15 with reference to the beam diameter d of the laser beam emitted from the laser light-source or of the semiconductor laser beam formed into a parallel beam. Also, executing the doubling makes it possible to reduce the adjacent beam spacing further down to $1/2^{M'}$. Namely,

20 without using the lens system, it is possible to form the multi-spot lights having the pitch smaller than the diameter d of the laser beam formed into the parallel light-flux.

As a method of forming the above-described

25 plurality of N laser beams, there is provided one or more of 2nd beam-doubling prisms having a cross section of a triangle and a cross section of a parallelogram. Here, the triangle has an oblique side forming an angle

of substantially 45 degrees toward the light-emitting surface, and the parallelogram is in contact with this oblique plane and has a total reflection plane that is parallel to this oblique plane. This method, through

5 the operation of the second beam-doubling prisms, makes it possible to form, for example, N/2 laser beams into the plurality of N laser beams.

Moreover, there are used two or more types of the 2nd beam-doubling prisms having dimensions that are

10 different from the dimension of the above-described 2nd beam-doubling prism. Then, these prisms are arranged from the largest to the smallest in sequence from the travelling directions of the beams in an n-stage cascaded manner, thereby 2^n times multiplying the number

15 of the beams. Namely, assuming that the dimension of the beam-doubling prisms at the final stage is equal to D, their number is set to be N/2, and the dimension and the number of the beam-doubling prisms at a stage preceding thereto are set to be 2D and N/4, respec-

20 tively, and the dimension and the number of the beam-doubling prisms at the first stage are set to be $2^{n-1}D$ and $N/2^n$, respectively. This method allows the N laser beams to be obtained from an extremely small number of $N/2^{n+1}$ laser beams.

25 The methods explained so far permits the multi-spot lights to be obtained from the laser beams emitted from the one laser light-source or the plurality of laser light-sources. What is more, using

the polarization components or the beam-splitting
prisms, one beam is doubled in any one of the beam-
doubling methods. On account of this condition, an
energy summation of the beams after being split into

5   the two can be made equal to 92% or more of the
original beam energy. Besides, even when taking into
consideration the reflection losses on the surfaces of
the lenses forming the converging laser beams or on the
surfaces of the polarization components, 70% or more of

10 the total energy resulting from summing up the respec-
tive emitting laser energies can be converted into the
total energy of the multi-spot lights.

Furthermore, the reflection losses on the
surfaces of the above-described optical components are

15 prevented using an antireflection coat and the like.
This method makes it possible to convert, into the
total energy of the multi-spot lights, 90% or more of
the total energy resulting from summing up the respec-
tive emitting laser energies of the laser beams emitted

20 from the one or more of laser light-sources.

In comparison with the conventional method by
which the multi spots are formed from the intensities
at the various locations of the Gaussian distribution,
the splitting of the beam can be made uniform more

25 easily, and the variation in the respective spot
energies among the multi-spot lights can be restrained
within ±20%.

What is more, polarization states of the

lights incoming into the above-described polarization
components are adjusted to the correct states, or a
film thickness of a multi-layered film type polariza-
tion beam splitter is adjusted to the correct thick-

5 ness. This allows the intensities of the doubled beams
to be substantially equated with each other, thereby
making it possible to restrain within $\pm 10\%$ the
variation in the respective spot energies among the
multi-spot lights.

10 The above-explained $2^{M'}$ times multiplication
of the number of the laser beams makes it possible to
form the proximate multi microscopic spots. Thus, the
multi-spot lights obtained in this way are used for the
confocal point detection, the fluorescent light detec-

15 tion or the DNA detection or the like. This makes it
possible to execute the conventionally impossible
detection where the light utilization efficiency is
high and the variation among the multi-spot lights is
small, thereby eventually allowing the confocal point

20 detection, the fluorescent light detection or the DNA
detection or the like to be executed at a high-speed
and with a high-accuracy.

The multi-spot lights formed using the above-
described multi-spot light forming methods according to

25 the present invention are projected onto an object to
be observed. Then, the lights having been reflected at
or having passed through the object to be inspected are
caused to pass through multi apertures located at

positions at which the above-described projected spots are image-formed. Next, the lights having passed through the respective apertures are each detected individually. Executing the confocal point detection

5   in this way makes it possible to execute the confocal point detection where the light utilization efficiency is high and the variation among the multi-spot lights is small, thereby implementing the high-speed and high-accuracy confocal point detection.

10   The multi-spot lights formed using the above-described multi-spot light forming methods according to the present invention are employed as excitation lights, then being projected onto the object to be observed that includes a fluorescent material. Then,

15 fluorescent lights generated by the excitation lights at the object to be inspected are separated from the excitation lights. Next, detectors are located at positions at which the fluorescent lights generated at the excitation light-projected spots are image-formed,

20 thereby detecting the fluorescent lights at the positions of the respective multi-spot lights. This allows the plurality of positions to be simultaneously irradiated with the excitation lights with a high-intensity. Also, since the variation among the respec-

25 tive spots is small, it becomes possible to execute a high-speed and high-accuracy fluorescent light detection.

In the above-described fluorescent light

detection, the fluorescent lights are caused to pass
through multi apertures located at the positions at
which the fluorescent lights generated at the excita-
tion light-projected spots are image-formed. In
5 addition, there are located the detectors for individ-
ually detecting the fluorescent lights having passed
through the respective apertures. This eliminates
fluorescent lights other than the fluorescent lights
generated by the excitation lights at the object to be
10 observed, thereby making it possible to execute a
fluorescent light detection having a high signal-to-
noise ratio.

 The multi-spot lights formed using the above-
described multi-spot light forming methods according to
15 the present invention are employed as the excitation
lights, then being projected onto the object to be
observed that includes a fluorescent material-added
DNA. The target to be observed is implemented in the
following way: A known probe DNA is attached to a
20 predetermined position on a substrate such as a glass,
and a target DNA is poured onto the glass substrate so
as to hybridize the target DNA corresponding to the
nucleotide sequence of the probe DNA, thus implementing
the object to be observed. Here, the target DNA is
25 formed by adding the fluorescent material to an end of
the DNA purified and amplified from a living body
sample, i.e., a target to be inspected. The excitation
lights of the multi-spot lights are irradiated with the

object to be observed so as to excite the fluorescent
material added to the DNA, thereby generating fluo-
rescent lights. Then, the fluorescent lights are
separated from the excitation lights, and the detectors

5   are located at positions at which the fluorescent
lights generated at the excitation light-projected
spots are image-formed. This makes it possible to
detect the fluorescent lights at the positions of the
respective multi-spot lights, thus allowing the DNA to

10 be inspected from the detected positions and the
detected signal intensity.

In the above-described DNA inspection, the
fluorescent lights are caused to pass through the multi
apertures located at the positions at which the fluo-

15 rescent lights generated at the excitation light-
projected spots are image-formed, then individually
detecting the fluorescent lights having passed through
the respective apertures. This makes it possible to
detect the fluorescent light intensities at the

20 positions of the respective multi-spot lights so as to
inspect the DNA, thereby being capable of executing the
DNA inspection having an even higher signal-to-noise
ratio.

In the fluorescent light detection and the

25 DNA inspection, in many cases, a wavelength of the
excitation lights and that of the fluorescent lights
come comparatively close to each other. The use of the
multi-spot lights in such a case enlarges the fields-

of-view in the detection and the inspection. This condition makes insufficient the characteristics of the optical systems for separating the fluorescent lights from the excitation lights, the optical systems being

5 inserted halfway in the course of guiding, to the detecting systems, the fluorescent lights generated by the respective multi-spot excitation lights. Namely, the incident angles of the fluorescent lights incoming into the optical systems for separating the fluorescent

10 lights from the excitation lights become different at the respective spots. Here, examples of the optical systems are the wavelength separating beam splitter and the interference filter that are installed halfway extending from the image-forming optical system to the

15 detectors. As a result, the excitation lights to be shielded leak away depending on the location in the multi-spot lights, which makes the accurate detection impossible.

In order to solve the above-described

20 problem, in the method of separating the fluorescent lights from the excitation lights, in addition to still using the wavelength separating beam splitter, the image-forming optical system is replaced by a telecentric image-forming optical system. In the

25 telecentric image-forming optical system, primary light-rays of the fluorescent lights become parallel to each other, the fluorescent lights being generated from the respective spots extending from the image-forming

optical system to the image-formed positions through the excitation by the respective multi-spot lights. Moreover, the interference filter and/or the wavelength separating beam splitter are inserted between the

5 telecentric image-forming optical system and the image-forming positions. This method causes the fluorescent light generated from whatever spot excitation light to be launched into the interference filter or the wavelength separating beam splitter at an incident angle

10 that is common to all the fluorescent lights. As a consequence, concerning the fluorescent light from whatever spot and the excitation lights becoming the noise components as well, it becomes possible to select the fluorescent lights and to eliminate the excitation

15 lights under the same condition, i.e., under an optimum condition. This situation allows the fluorescent light detection to be executed with an extremely small amount of the excitation light leakage, i.e., with an extremely small amount of the noise. As is seen from

20 the above-described explanation, the use of this fluorescent light detecting method further makes it possible to perform, precisely and with a high-accuracy, the DNA inspection where it is required to detect the fluorescent lights the intensities of which

25 are exceedingly weaker as compared with the intensities of the reflected excitation lights.

It is an object of the present invention to provide a distribution measuring apparatus for measur-

ing the fluorescent marker distribution in the DNA
probe array and the like, the distribution measuring
apparatus being capable of solving the conventional
problems and accomplishing the high-resolution

5 performance and the high-speed characteristic at the
same time.

The fluorescent marker distribution measuring
apparatus according to the present invention and
accomplishing the above-described object is an

10 apparatus for scanning and reading a light-emission
pattern from the fluorescent marker captured on a
sample surface on a substrate. The fluorescent marker
distribution measuring apparatus is characterized by
including the following configuration components: A

15 stage for mounting thereon the substrate of a target to
be read, a unit for forming a plurality of irradiation
spots on the sample surface on the substrate with a
specified spacing that is larger than a diameter of the
irradiation spots, a movement mechanism for moving the

20 stage, a converging/detecting unit for converging and
detecting light-emissions generated from the plurality
of irradiation spots on the sample surface, a control
unit for controlling the movement mechanism so that the
substantially entire surface of the sample surface is

25 irradiated in correspondence with the formation
positions of the plurality of irradiation spots, and a
processing unit for processing a signal from the
converging/detecting unit so as to reconstruct images

on the sample surface. The intensities of the plural-
ity of irradiation spots are set to be substantially
almost equal to each other.

     The unit for forming the plurality of
5   irradiation spots can be configured as follows: The
unit includes at least one laser light-source as
excitation light-sources. Moreover, the unit splits a
light emitted from an excitation light-source so as to
image-form the irradiation spots in such a manner that
10 the irradiation spots are arranged on the sample
surface and a spacing of adjacent irradiation spots
becomes smaller than 1mm. The unit for splitting the
light emitted from the excitation light-source can be
configured as the splitting prism, the optical aniso-
15 tropic medium, the optical fiber, a polarization mirror
or a combination of these devices.

     The unit for forming the plurality of
irradiation spots can also be configured as follows:
The unit includes N (N$\geq$1) excitation light-sources and
20 M-stage 2-splitting units (M>2), and forms N$\times$2^M
irradiation spots. As the 2-splitting unit, there can
be employed the splitting prism, the optical aniso-
tropic medium, the optical fiber or the polarization
mirror.

25   The unit for forming the plurality of
irradiation spots can also be configured as follows:
The unit includes a plurality of laser light-sources
and a plurality of optical fibers. Furthermore, light-

emitting ends of the plurality of optical fibers are located on a straight line, thereby forming the plurality of irradiation spots.

It is preferable that the plurality of
5   irradiation spots should be positioned on a straight line with a substantially fixed spacing.

The converging/detecting unit converges and, almost simultaneously, detects a plurality of fluorescent emitted lights generated from the plurality of
10   irradiation spot-portions. The unit includes a wavelength selecting filter so as to separate fluorescent components from excitation components out of the emitted lights generated from the plurality of irradiation spot-portions, then detecting the fluorescent
15   components. The converging/detecting unit can also be configured as follows: The unit has pinholes at which the emitted lights generated from the plurality of irradiation spot-portions are image-formed, then detecting the lights passing through the pinholes, the
20   pinholes the number of which being the same as that of the irradiation spot-portions.

The control unit makes it possible to measure the entire plane of the sample surface by the following method: Employing, as a unit, step-like movements on a
25   first microscopic width basis at n times and a step-like movement on a second width basis at one time subsequent thereto, the second width being greater in comparison with the first microscopic width, the stage

is displaced in a specified one-axis direction. Then,
this process is repeated, thereby scanning the sample
surface. More concretely, the control unit is capable
of controlling the stage movement so that the following

5   operation is implemented: Employing, as the unit,
(n-1)-times step-like style movements on the basis of a
width of 1/n (n: an integer) of the irradiation spot
spacing and the subsequent one-time step-like style
movement on the basis of (a width between both ends of

10 the excitation spot group + the first width), the
movement in the irradiation spots-arranged direction is
executed, and this process is repeated thereby scanning
the sample surface.

It is desirable that fetching of the signal

15 from the converging/detecting unit should be executed
while the stage is being displaced in a direction
perpendicular to the plurality of irradiation spots-
arranged direction.

The unit for forming the plurality of

20 irradiation spots is required to form the plurality of
irradiation spot-portions simultaneously. In order to
image-form the lights onto the sample surface, there is
employed a condenser lens the data of which is as
follows: The numerical aperture is 0.7 or more, the

25 effective field-of-view is 1.0 mm or more, and the
difference between the sample surface and the lens tip
is 0.7 mm or more.

The unit for forming the plurality of

irradiation spots can also be configured as follows:
The unit includes a plurality of optical anisotropic
media the lengths of which in the optical axis direc-
tion differ from each other or a plurality of optical
5 anisotropic media the crystalline-axis directions of
which differ from each other, and a plurality of
wavelength plates located between the adjacent optical
anisotropic media.

The unit for forming the plurality of
10 irradiation spots can also be configured as follows:
The unit includes a device where the plurality of
optical anisotropic media and the plurality of wave-
length plates are located alternately. Moreover, a
length of each optical anisotropic medium in the light-
15 travelling direction is equal to about one-half of a
length of an optical anisotropic medium located
immediately preceding thereto.

The unit for forming the plurality of
irradiation spots may also be configured as follows:
20 The lights from the excitation light-sources are
scanned and launched in sequence into one ends of a
plurality of aligned optical fibers, and light-emitting
ends of the optical fibers are located on a straight
line, thereby forming the plurality of irradiation
25 spots.

Also, it is preferable that there should be
provided a function of executing an intensity correc-
tion for each detection signal of the respective

irradiation spots.

It is an object of the present invention to provide a technology for detecting weak fluorescent lights at a high-speed, with a high-resolution and with

5 a high-sensitivity with reference to a target to be detected that requires 2-dimensional large number of lines of resolution. Also, It is another object thereof to provide, using this technology, a technology for inspecting a DNA with a high-sensitivity and at a

10 high-speed.

In order to accomplish the above-described objects, the present invention employs the following methods and units:

The target object having a fluorescent

15 characteristic is irradiated with multi-spot excitation lights including a large number of M microscopic spots. Fluorescent lights generated by the excitation lights from the respective multi spots are separated from the excitation lights. Then, fluorescent light images

20 emitted from the target object are detected using a plurality of weak light detecting devices allowing the photon counting to be executed. This makes it possible to detect even an extremely weak fluorescent light. Photon signals obtained from the respective detecting

25 devices are each photon-counted individually, then individually storing the photon-counted numbers Npm detected by the respective detecting devices. In addition, positions of the multi-spot lights and a

position of the target object are changed relatively using a driving system and the like, then storing in sequence the photon-counted numbers from the respective detectors. Storing and collecting in this way the

5 photon-counted number data over a desired range on the target object makes it possible to construct the fluorescent light picture from the collected data.

Also, sheet-shaped excitation lights are used instead of the above-described multi-spot lights.

10 Fluorescent lights, which have been generated from irradiation areas obtained by the irradiation with the sheet-shaped excitation lights and having a long and narrow configuration, are separated from the excitation lights. Then, fluorescent light images emitted from

15 the target object are detected using the plurality of weak light detecting devices allowing the photon counting to be executed. Photon signals obtained from the respective detecting devices are each photon-counted individually, then individually storing the photon-

20 counted numbers Npm detected by the respective detecting devices. In addition, positions of the irradiation areas resulting from the sheet-shaped excitation lights and having the long and narrow configuration and the position of the target object are changed relatively,

25 then storing in sequence the photon-counted numbers from the respective detectors. Storing and collecting in this way the photon-counted number data over a desired range on the target object makes it possible to

construct the fluorescent light picture from the collected data.

By executing the irradiation with the multi-spot lights or the sheet-shaped excitation lights and detecting the fluorescent lights from M pixels simultaneously, it becomes possible to spend a time of M to 2M μsec per pixel in the case of trying to detect the 6000×6000 pixels in 1 minute. Here, the case has been described previously in the conventional problems. For example, assuming M = 50, this time becomes equal to 50 to 100 μsec. This condition makes it possible to solve the previously-described problem concerning the delay in the fluorescent light generation. Also, the following reason permits the dynamic range to be ensured and the high-sensitivity detection to be executed.

Namely, the previously-described per-pixel detecting time of the order of 1 μsec shortens a detecting time that is long enough for a photo counting measuring time needed to execute the high-sensitivity detection, thereby making it difficult to ensure the dynamic range. This is attributed to the fact that, since the pulse-width of a photon pulse signal generated when one photon is detected is equal to tens of n seconds, only a signal of, at most, tens of pulses can be detected within the time of 1 μsec. Also, even if, in such a short time, an attempt is made to detect the fluorescent light intensity in an analogue manner,

frequency characteristics of the detecting circuit make it difficult to execute the detection in a state where a sufficient dynamic range has been ensured.

Employing, as the above-described target
5 object, a sample obtained from coupling a fluorescent molecule-added DNA fragment with a DNA corresponding thereto, the inspection is executed in the following way: In the case where the target to be inspected is, as an example, a DNA chip, a target that results from
10 adding a desired fluorescent material to a DNA fragment formed from the target to be inspected, i.e., a DNA, is hybridized with the DNA chip at first. Then, the hybridized DNA chip to be inspected is irradiated with the multi-spot excitation lights including the large
15 number of M microscopic spots. Fluorescent lights obtained by the irradiation from the multi spots are separated from the excitation lights. Next, separated fluorescent light images emitted from the DNA chip are detected using the plurality of weak light detecting
20 devices allowing the photon counting to be executed. Moreover, photon signals obtained from the respective detecting devices are each photon-counted individually, then individually storing the photon-counted numbers Npm detected by the respective detecting devices. In
25 addition, positions of the multi-spot lights and a position of the DNA chip are changed relatively, then storing in sequence the photon-counted numbers from the respective detectors. In this way, the photon-counted

number data is stored and collected over a desired range on the DNA chip so as to construct the fluorescent light picture from the collected data, thereby inspecting the DNA.

5   The use of the sheet-shaped beams instead of the multi-spot lights also makes it possible to execute the DNA inspection by detecting the fluorescent material added to the target DNA in much the same way as explained in the above-described fluorescent light

10   detection using the sheet-shaped beams.

A multi-spot aperture or a long and narrow aperture that permits only a multi-spot image or a sheet-shaped long and narrow area image to pass through is located at a focal point achieving position of

15   fluorescent light images from the above-described target object surface, thereby performing the confocal point detection, the focal point achieving position being situated at a position at which the diameters of the multi-spot excitation lights or the sheet-shaped

20   excitation lights with which the target object is irradiated become minimum-focused diameters. This makes it possible to obtain the confocal point image having a high signal-to-noise ratio. Namely, it becomes possible to detect, without being subjected to

25   the influence from a fluorescent light noise and thus with a low-noise, the fluorescent lights at the multi-spot positions to be detected or the fluorescent lights at the sheet-shaped beam positions to be detected.

Here, the fluorescent light noise exists in the back-
ground of substances such as a fluorescent material
existing in optical paths other than the above-
described positions to be detected. If the sample

5   toward which the fluorescent light detection is
performed exists within a 2-dimensional plane, relative
positions between the multi spots and the sample are
changed within the plane, then performing the confocal
point detection. This method allows the high signal-

10 to-noise ratio fluorescent light detection or DNA
inspection to be executed in a state of being scarcely
subjected to the influence of a noise by a fluorescent
material existing outside the plane.

In the above-described fluorescent image

15 detection using the multi-spot lights, the relative
positions between the multi spots and the sample are
changed in at least one direction out of three
directions in total, i.e., two directions within the
target object surface and the remaining one direction

20 perpendicular to the target object surface. In
particular, in the case of the above-described confocal
point detection, the relative position of the target is
changed in the optical axis direction from the position
at which the diameters of the multi spots or the sheet-

25 shaped beams become the minimum-focused diameters, then
determining the focal point achieving position from the
obtained variation in the fluorescent light image
intensity. Accordingly, from the fluorescent light

intensity at this position and toward a target having irregularity on its surface, it becomes possible to obtain the accurate fluorescent light intensity information, surface irregularity information, or informa-

5 tion on the 3-dimensional positions emitting the fluorescent lights.

As having been pointed out in the problem in the case of the above-described conventional one-spot excitation light, the larger the number of the multi-

10 spot lights becomes, the more advantageous it becomes to detect the fluorescent light image at a high-speed. Setting M to be 10 or larger increases, up to hundreds, the maximum photon-counted number detectable within this time, thereby becoming effective in implementing

15 the high-sensitivity characteristic and the broad dynamic range. Since an even higher-speed character- istic is implemented in the DNA inspection and the like, providing the plurality number of spots exhibits an outstanding effect. Making M equal to 50 or more

20 exhibits a more outstanding effect. Namely, setting M to be 50 or larger makes it possible to detect, for example, the 6000×6000 pixels in spending tens of μ seconds per pixel, thereby allowing all the pixels to be detected within 1 minute.

25 The above-described multi spots are arranged on a 1 or 2-dimensional straight line in order to accomplish the following purposes: Detecting the multi spots using a plurality of detecting devices, above

all, a plurality of detecting devices arranged on a 1 or 2-dimensional straight line, and causing the detected plural pieces of information to correspond to the position information of the target or the DNA chip

5   so as to output the detected information as the fluo-rescent light image.

The relative positions between the multi-spot excitation lights or the sheet-shaped excitation lights and the target are changed, and a period during which

10 this movement by the amount of one pixel is executed is set to be Td. At this time, a value $\alpha Td/\Delta t_0$ is deter-mined as follows: First, a value $Td/\Delta t_0$ is determined by dividing the period Td by Δt_0. Here, Δt_0 denotes the pulse-width of the previously-described photon

15 counting signal at the time of detecting a single photon. Then, the value $\alpha Td/\Delta t_0$ is determined by multiplying $Td/\Delta t_0$ by a desired coefficient α that is larger than 0.1 and smaller than 1. Moreover, employ-ing, as a judging criterion, a condition that this

20 value has reached the above-described photon-counted number Npm, i.e., at the time of $\alpha Td/\Delta t_0 \geqq Npm$, the detection is executed in a state of weakening the fluorescent light detection intensities so that the photon counting becomes executable. This weakening is

25 performed using such a method as changing the inten-sities of the multi-spot excitation lights or the sheet-shaped excitation lights. Namely, in the point of the accuracy, it is impossible to execute the photon

counting that is more than α times as large as the value obtained by dividing the per-pixel detecting time by the photon pulse time-width. Accordingly, in the following way, the photon counting value is adjusted to

5 the value toward which the photon counting is effectively executable: Weakening the excitation lights, shortening the excitation light irradiation time to effectively lower the excitation light intensities, or lowering the gain of the detecting device so as to weak

10 the detected fluorescent lights.

The above-described multi-spot excitation lights or sheet-shaped excitation lights are converted into multi-color lights having 2 or more of wavelengths. This increases the information on the

15 fluorescent light detection, thereby enhancing the fluorescent light detecting function and the DNA detecting function.

Also, a time during which the above-described photon pulse signal is being positioned at a high level

20 is addition-measured during a time βTd that is less than the per-pixel detecting time Td. When setting the value of this time to be Th', it is judged whether or not a condition Th' $\geqq$ $\gamma \beta Td$ is satisfied with reference to a predetermined γ that is smaller than 1.

25 If this inequality should be satisfied, there is employed a result obtained by integrating the output signal from the detecting device in an analogue-style integrating circuit. This makes it possible to perform

the detection toward stronger fluorescent lights as well. Namely, even in the case of detecting the strong fluorescent lights the photon counting of which is impossible, executing the analogue-style integration

5 allows the strong lights to be detected. What is more, making the analogue-style detecting time equal to Td or less allows all the pixels to be detected without changing the per-pixel detecting time. As a conse- quence, it becomes possible to execute the detection

10 with a high-resolution and at a high-speed over a broad dynamic range ranging from the weak fluorescent lights necessitating the photon counting to the strong detec- tion intensities necessitating the analogue integrating detection based on the above-described integrating

15 circuit. Also, as a detecting method where the above- described α is equivalent to 1, there exists the following method: The photon counting and the analogue integrating detection are executed in parallel simul- taneously so as to compare and judge both results

20 obtained, then employing one of the detected results.

Also, a laser light-source is used as the above-described excitation light-source. A multi-spot generating hologram or a sheet-shaped beam generating hologram is located within the laser resonator, and the

25 multi spots are generated using the strong laser light within the laser resonator. Usually, the optical energy within the laser resonator is more than 10 times as high as the emitting beam energy. In an ordinary

laser resonator, the reflectance of a mirror at the emitting window is set to be substantially 90%, and the remaining substantially 10% is extracted as the emitting light. The reflectance at the emitting window is

5   made equal to 100% so as to confine the light within the resonator, and the hologram is located in an optical path within the resonator. This method allows substantially 10% of the hologram' diffraction efficiency to be utilized as the multi-spot lights or

10 the sheet-shaped beams. As a result, it is possible to obtain the multi-spot lights or the sheet-shaped beams the intensities of which are almost 10 times higher as compared with those in the conventional case where the hologram is located in a laser-emitting optical path.

15 Consequently, it becomes possible to execute the high-speed and high-sensitivity fluorescent light detection and DNA inspection.

The present invention permits a laser light emitted from a laser light-source to be converted into

20 the multi-spot lights with a exceedingly high-efficiency and without being wasted. At the same time, the present invention makes it possible to extremely lessen the variation among the respective multi-spot lights. As a consequence, using the multi-spot lights,

25 it becomes possible to execute the high-speed and high-accuracy confocal point detection. Also, using the multi-spot lights, it becomes possible to execute the high-speed and high-accuracy fluorescent light detec-

tion and DNA inspection.

The present invention allows even the analogue integrating detection to be executed, thereby making it possible to execute the detection having a

5   exceedingly broad dynamic range. Namely, it becomes possible to even detect the fluorescent light image of, for example, the 6000×6000 pixels in the dynamic range of 2^{16} in the detecting time of 1 minute. As a result, it becomes possible to detect targets to be inspected,

10   such as the DNA chip, in the detecting time of an order of 1 minute. This eventually makes it possible to detect, in a short time and precisely, a large quantity of samples to be inspected that are collected in a widely executed group checkup.

15   Also, the present invention is not only applied to the DNA inspection, but is widely used for a fluorescent light detecting microscopic method. The present invention tremendously shortens the times that have been required for the conventional observations

20   and inspections. Namely, it becomes possible to detect, in a short time, even a weak light toward which only several photons can be detected. Also, it becomes possible to execute, in a short time and with a high-accuracy, the detection over a broad range ranging from

25   the weak light to the strong light to be detected.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a diagram for illustrating an

embodiment in the present invention;

FIG. 2 is an embodiment-illustrating diagram in the present invention, which indicates multi-spot irradiation;

5   FIG. 3 is an embodiment-illustrating diagram in the present invention, which indicates the movement of the multi-spot irradiation;

FIG. 4 is an embodiment-illustrating diagram in the present invention, which indicates multi-spot

10   generation by a microlens array;

FIG. 5 is an embodiment-illustrating diagram in the present invention, which is a diagram for explaining the embodiment in FIG. 1;

FIGS. 6A, 6B, 6C, 6D, 6E are diagrams for

15   explaining the movement of spot fluorescent light images at the time of fixing a wavelength selection beam splitter;

FIG. 7 is a diagram for explaining the movement of the spot fluorescent light images at the

20   time of deflecting the wavelength selection beam splitter;

FIGS. 8A to 8F are diagrams for illustrating operations, detection signals and so on of the respective components in FIG. 1;

25   FIG. 9 is a diagram for illustrating the movement of the images on a fluorescent light image detection plane in the embodiment in FIG. 1;

FIG. 10 is a diagram for illustrating light-

receiving apertures on the fluorescent light detection plane in the embodiment in FIG. 1;

FIG. 11 is an embodiment-illustrating diagram in the present invention, where the multi spots are

5 formed using a hologram;

FIG. 12 is a diagram for illustrating a method of creating the hologram in FIG. 11;

FIG. 13 is a diagram for illustrating another embodiment in the present invention;

10 FIG. 14 is a diagram for illustrating a 2-dimensional multi-spot generating microlens array used in the embodiment in FIG. 13;

FIG. 15 is a diagram for indicating a characteristic of the wavelength separating beam

15 splitter in the embodiments in the present invention;

FIG. 16 is a diagram for indicating a characteristic of an interference filter in the embodiments in the present invention;

FIG. 17 is an embodiment-illustrating diagram

20 in the present invention, where excitation lights are light-shielded using a space filter;

FIG. 18 is a diagram for illustrating the space filter in FIG. 17;

FIG. 19 is another embodiment-illustrating

25 diagram in the present invention, which is a diagram for illustrating an excitation light light-shielding method utilizing the polarization;

FIG. 20 is an embodiment-illustrating diagram

in the present invention, where the 1-dimensional spot array is detected using a 2-dimensional multi-channel photomultiplier tube;

FIG. 21 is a diagram for illustrating light-receiving and light-emitting ends of optical fibers and taking-in of spot images in the embodiment in FIG. 20;

FIG. 22 is an embodiment-illustrating diagram in the present invention, where the detection is executed using excitation lights having a plurality of different wavelengths;

FIG. 23 is an embodiment-illustrating diagram in the present invention, where a plurality of semiconductor lasers are used as the excitation lights;

FIG. 24 is an embodiment-illustrating diagram in the present invention, where multi-spot lights are obtained from light-emitting ends of optical fibers;

FIG. 25 is an embodiment-illustrating diagram in the present invention, where the multi-spot lights are obtained from one laser beam;

FIG. 26 is an embodiment-illustrating diagram in the present invention, where using excitation lights having a plurality of different wavelengths, the simultaneous detection is executed;

FIG. 27 is an embodiment-illustrating diagram in the present invention, where irradiation with the multi-spot lights is performed in an oblique direction and regularly reflected excitation lights are light-shielded on a pupil;

FIG. 28 is a diagram for illustrating a light-shielding portion on the pupil in FIG. 27;

FIG. 29 is an embodiment-illustrating diagram in the present invention, which indicates a method of

5 detecting scattered excitation lights so as to determine a foreign substance position and correcting a fluorescent light detection result;

FIG. 30 is a diagram for illustrating the regularly reflected excitation lights and the scattered

10 excitation lights between an objective lens and a DNA chip in FIG. 29;

FIGS. 31A, 31B, 31C are diagrams for indicating a method of the correction by a detection signal of the foreign substance-scattered excitation lights;

15 FIG. 32 is an embodiment-illustrating diagram in the present invention, which indicates focus detection and the control;

FIG. 33 is a diagram for illustrating a reflection response of the DNA chip in the focus

20 detection in FIG. 32;

FIG. 34 is an embodiment-illustrating diagram in the present invention, where the focus detection is executed through the objective lens;

FIG. 35 is an embodiment-illustrating diagram

25 in the present invention, which indicates a method of detecting the entire inspection target area of the DNA chip;

FIG. 36 is an enlarged explanatory diagram

for explaining the embodiment in FIG. 35;

FIG. 37 is an enlarged explanatory diagram for explaining the embodiment in FIG. 35;

FIG. 38 is a diagram for indicating the move-
5 ment of the multi spots in the embodiment in FIG. 35;

FIG. 39 is a diagram for indicating the move-
ment of a DNA chip chuck in the x direction in the
embodiment in FIG. 35;

FIG. 40 is a diagram for indicating the move-
10 ment of the DNA chip chuck in the y direction in the
embodiment in FIG. 35;

FIG. 41A is a front view for illustrating the
configuration of an embodiment of the multi-spot light
forming unit according to the present invention;
15 FIGS. 41B, 41C, and 41D are partially
enlarged diagrams of FIG. 41A;

FIG. 42 is a front view for illustrating the
configuration of an embodiment of the multi-spot light
forming unit according to the present invention;
20 FIG. 43 is a front view for illustrating an
embodiment of a mask according to the present
invention;

FIG. 44 is a front view for illustrating the
configuration of an embodiment of the multi-spot light
25 forming unit according to the present invention;

FIG. 45 is a perspective view for illustrat-
ing the configuration of an embodiment of the fluo-
rescent light detecting and DNA inspecting apparatus

according to the present invention;

FIG. 46 is a front view for illustrating an embodiment of the multi-spot light forming unit according to the present invention;

5 FIG. 47 is a front view for illustrating an embodiment of a 2-dimensional multi-spot light forming unit according to the present invention;

FIG. 48 is a front view for illustrating an example of the arrangement of the 2-dimensional multi-

10 spot lights according to the present invention;

FIG. 49 is a front view for illustrating an embodiment of a confocal point detecting apparatus according to the present invention;

FIG. 50 is a diagram for indicating the

15 relationship between the multi-spot lights and stage scanning in the confocal point detection according to the present invention;

FIG. 51 is a schematic diagram for illustrating the entire configuration of one embodiment of a

20 fluorescent marker distribution measuring apparatus according to the present invention;

FIG. 52 is a diagram for illustrating the configuration of an embodiment of a multi light-source distribution unit according to the present invention;

25 FIG. 53 is a diagram for illustrating the configuration of another embodiment of the multi light-source distribution unit according to the present invention;

FIG. 54 is a diagram for illustrating the configuration of another embodiment of the multi light-source distribution unit according to the present invention;

5 FIG. 55 is a diagram for illustrating the configuration of another embodiment of the multi light-source distribution unit according to the present invention;

FIG. 56 is a diagram for illustrating the configuration of another embodiment of the multi light-source distribution unit according to the present invention;

FIG. 57 is a diagram for illustrating the configuration of another embodiment of the multi light-source distribution unit according to the present invention;

FIG. 58 is a diagram for illustrating the configuration of an embodiment of a multi-detection unit according to the present invention;

20 FIG. 59 is a diagram for illustrating the configuration of another embodiment of the multi-detection unit according to the present invention;

FIG. 60 is a diagram for illustrating the configuration of another embodiment of the multi-detection unit according to the present invention;

25 FIG. 61 is a diagram for illustrating the configuration of another embodiment of the multi-detection unit according to the present invention;

FIG. 62 is a diagram for illustrating the configuration of another embodiment of the multi-detection unit according to the present invention;

FIG. 63 is a diagram for illustrating the configuration of another embodiment of the multi-detection unit according to the present invention;

FIG. 64 is a diagram for illustrating an example of images detected;

FIG. 65 is an explanatory diagram for explaining an example of the displacement of an XYZ driving unit 20f;

FIGS. 66A, 66B are diagrams for illustrating displacement curves of x, y axes of the XYZ driving unit 20f;

FIG. 67 is a diagram for illustrating the configuration of another embodiment of the multi light-source distribution unit according to the present invention;

FIG. 68 is a diagram for illustrating the configuration of another embodiment of the multi light-source distribution unit according to the present invention;

FIG. 69 is a diagram for illustrating the configuration of another embodiment of the multi light-source distribution unit according to the present invention;

FIG. 70 is a perspective view for illustrating the schematic configuration of the DNA chip

inspecting apparatus according to the present

invention;

FIG. 71 is a plane view for indicating the

relationship between cells of the DNA chip and the

5   excitation lights;

FIG. 72 is a diagram for explaining the

scanning of the DNA chip in the x, y directions accord-

ing to the present invention;

FIG. 73 is a diagram for indicating the

10   relationship between the scanning of the DNA chip in

the x direction and photon detection according to the

present invention;

FIG. 74 is a diagram for illustrating photon

counting signals of weak lights;

15   FIGS. 75A to 75D are diagrams for illustrat-

ing photon pulse signals in the case of strong detected

lights;

FIG. 76 is a block diagram for illustrating a

circuit configuration for detecting a broad dynamic

20   range according to the present invention;

FIGS. 77A to 77E are diagrams for indicating

a method of differentiating between the weak detected

lights and the strong detected lights according to the

present invention;

25   FIGS. 78A, 78B are diagrams for indicating a

method of detecting even stronger detected lights

according to the present invention;

FIG. 79 is a plane view of the cells of the

DNA chip for illustrating the case where there are employed the sheet-shaped excitation lights according to the present invention; and

FIG. 80 is a perspective view of a portion of 5 an optical system for illustrating an embodiment where the multi spots according to the present invention are formed by a hologram within a laser resonator.

DESCRIPTION OF THE EMBODIMENTS

FIG. 1 is a diagram for illustrating an 10 embodiment in the present invention. The reference numerals denote the following configuration components: 1 A multi-spot excitation lights-irradiating system for forming multi-spot excitation lights to irradiate a DNA chip 2 with the excitation lights so that a fluo- 15 rescent light detection can be executed, 3 a fluo- rescent light detecting system for detecting fluo- rescent lights generated by the multi-spot excitation lights, 11 an excitation light-source including an excitation light light-source and an excitation light 20 beam forming optical system. A He-Ne laser light beam is formed into a desired longitudinal-to-transverse ratio of the beam diameter by two cylindrical lenses having different focal lengths, then being launched into an AO deflector 12 through a mirror 1000. The AO 25 deflector 12 includes a terminal of a frequency-ω high- frequency voltage applied to a quartz oscillator, and an input terminal of an amplitude signal the frequency

ω_v of which is lower than the frequency ω.

The signal with the frequency ω, which is transmitted from a control apparatus 4, has a frequency band in a range of $\omega \pm \omega_0$. Changing the frequency

5 results in a change in the diffraction angle of the excitation light incoming into the AO deflector 12. Also, an inputting of the amplitude signal ω_v from the control apparatus 4 brings about a change in the diffraction efficiency, thereby making it possible to

10 control the diffraction light intensity. The diffraction light having passed through the AO deflector is separated from its 0th light (not illustrated) (the 0th light is light-shielded). Then, a lens system 13 including two lenses 131, 132 of focal lengths f_1, f_2

15 irradiates a microlens array 14 with the separated diffraction light in a desired beam diameter. Changing the frequency of the AO deflector 12 changes the angle of the excitation light incoming into the microlens array 14, but causes no change in the position thereof.

20 FIG. 4 is an enlarged detailed view of the microlens array 14. Microscopic microlenses composed of glass are arranged in a 1-dimesional configuration in a large number of 32 to 256. Let's explain the light incoming therein: For example, lights 1010

25 represented by solid-lines pass through the respective microlenses 141, 142, $\cdots \cdots$, then image-forming microscopic spots 111, 112, $\cdots \cdots$, 11M on a straight line ℓ on a focal point plane Σ. As illustrated by

dotted-lines in FIG. 4, changing the frequency of the AO deflector 12 changes the angle of the excitation light incoming into the microlenses. As a result, the positions of the microscopic spots on the straight line

5 l are changed to the positions as indicated by $1j_21$, $1j_22$, ..., $1j_2M$.

As is illustrated in FIG. 1, through the use of a lens 15 and an objective lens 16, a fluorescent material added to targets hybridized with the DNA chip

10 2 is irradiated and excited with the microscopic-spot array 111, 112, ..., 11M formed on the focal point plane Σ of the microlens array 14. The focusing of the objective lens 16 is achieved so that the minimum beam diameter is formed on a glass surface $\Sigma1$ or $\Sigma2$ to

15 which the targets have been attached (Concerning the details, refer to FIG. 33).

FIG. 2 illustrates the details of a surface structure of the DNA chip 2. Square-shaped minimum units 201, 211, 202, 212 and so on, which are indicated

20 by longitudinal and transverse thin lines, represent detection pixels. In the drawing, a cell 20 is equivalent to 5×5 pixels (indicated by a bold line). A fragment of the same DNA information is planted in one cell. Consequently, a target having the same DNA

25 fragment structure is hybridized with this cell.

The reason why one cell is divided into a plurality of pixels is as follows: When a foreign substance such as a protein is mixed in one cell, in

some cases, an excitation light incoming into this

protein generates a large-intensity fluorescent light.

The dimension of such a foreign substance is usually

equal to, at most, several μm. Accordingly, if the

5   pixel dimension is equal to several μm and the cell

dimension is equal to 10 μm for example, and if a

method as described later allows the foreign substance

position to be detected and be separated within the

cell of 10 μm, it becomes possible to precisely

10   determine the magnitude of the fluorescent light in

accordance with the information on a portion other than

the foreign substance portion.

FIG. 3 illustrates a side view of the DNA

chip. The configuration given in this drawing is that

15   the hybridized targets are mounted in a state of being

naked on a glass substrate. This state is presented in

some cases, and there are other cases where, as will be

described later, the hybridized targets are sandwiched

between glass substrates. In any case, the microscopic

20   excitation light spots are converged onto the plane on

which the targets exist. The light-converged diameter

is substantially equal to the dimension of the pixel to

be detected, i.e., the square-shaped minimum unit

illustrated in FIG. 2.

25   At the initial frequency of the AO deflector

12, there has been obtained the diffraction light

represented by solid-lines in FIG. 1. At this time, as

illustrated in FIG. 3, the pixels 201, 202, 203, ··· ···,

20M on the DNA chip are irradiated with the excitation
light spot array as indicated by 101, 102, 103, ...,
10M. This irradiation, as will be explained later,
allows fluorescent lights to be detected from the

5 respective pixels. The irradiating time of the excita-
tion light spots is equal to a time Δt (several to
hundreds of μseconds) that is larger than the fluo-
rescent light attenuation time. In the present embodi-
ment, the irradiating time is equal to 60 μs.

10   Moreover, after the lapse of 60 μs, changing
the frequency of the AO deflector 12 gives rise to the
change in the diffraction angle of the excitation
light. Then, as illustrated in FIG. 1 and FIG. 4,
lights $10j_20$ denoted by dotted-lines are launched into

15 the microlens array 14. As a result, as illustrated in
FIG. 3, the DNA chip is irradiated with the excitation
light spot array as indicated by 111, 112, 113, ...,
11M. This irradiation allows fluorescent lights to be
detected from pixels 211, 212, 213, ..., 21M. In this

20 way, the irradiation with the M multi spots is executed
in sequence with a shift on a pixel-pitch basis. When
the shifts by the amount of j pixels have been
finished, it turns out that the fluorescent lights by
the amount of jM pixels have been detected all.

25   Next, referring to FIG. 1, the explanation
will be given below concerning the embodiment of the
fluorescent light detection. A beam splitter 30
located between the lens 15 and the objective lens 16

is a wavelength separating beam splitter.   In the
present embodiment, the wavelength of the He-Ne laser
light from the excitation light-source is equal to 633
nm.   The fluorescent material added to the targets on
5   the DNA chip is Cy5, and the wavelength of the fluo-
rescent lights to be detected lies in proximity to 670
nm.

The wavelength separating beam splitter 30
permits the 45°-incident excitation light at the
10   wavelength of 633 nm to pass through by substantially
100%, and causes the 45°-incident fluorescent lights at
the wavelength of 670 nm to be reflected by substan-
tially 100%.   The beam splitter, however, reflects the
633 nm excitation light as well by a exceedingly slight
15   amount.   The exceedingly slight amount of reflection
becomes a problem since the fluorescent lights are
extremely weak.   Accordingly, in the embodiment in FIG.
1, an interference filter 34, which has its central
wavelength characteristic at 670 nm and the half-value
20   width of which is equal to about 15 nm, is inserted
into the fluorescent light detecting system 3 so as to
cause the interference filter to light-shield the
excitation light leakage.   Incidentally, the component
34 is not limited to the interference filter.   Thus, it
25   is allowable to use so-called a color filer for permit-
ting a certain value or more of wavelength light to
pass through and causing a certain value or less of
wavelength light to be light-shielded.   Also, the

combined use of the color filer and the interference
filter is allowable. Hereinafter, the explanation will
be given concerning the embodiments, using the inter-
ference filter alone for the simplicity of the explana-
5   tion.

Next, the explanation will be given below
concerning the following: Changes in the positions of
detected fluorescent light images when the positions of
the multi-spot excitation lights on the DNA chip are
10   changed using the AO deflector, and corrections of the
position changes needed in order to execute the detec-
tion with the fixed detector. The wavelength separat-
ing beam splitter 30 in FIG. 1 performs the corrections
of the position changes as well. FIG. 5 illustrates
15   the main portion in FIG. 1, and the same reference
numeral as that in FIG. 1 denotes the same component.
The wavelength separating beam splitter 30 is driven by
a piezo device 301 having a high resonant frequency
characteristic of 5 to 10 kHz, and is configured to
20   perform a microscopic rotation with the y axis as its
center.

FIGS. 6A to 6E and FIG. 7 are diagrams for
explaining the role of this microscopic rotation, and
the same reference numeral as that in FIG. 1 denotes
25   the same component. By the deflection signal inputted
into the AO deflector 12 from the control apparatus 4,
the respective positions of the multi excitation
microscopic spots 1011, 1021, ··· ···, 10M1 on the DNA

chip 2 in FIG. 6C are changed to 1012, 1022, ⋯ ⋯, 10M2

in sequence on the one-pixel basis. At this time,

assuming that the wavelength separating beam splitter

30 performs no rotation, as illustrated in FIG. 6D,

5   multi fluorescent light points 1211, 1221, ⋯ ⋯, 12M1

on a fluorescent light detection plane Σ_{PH} that is

located at a position conjugated with the DNA chip are

changed to 1212, 1222, ⋯ ⋯, 12M2 on the one-pixel

basis as well.

10   The fluorescent light spot image 1212 on the

fluorescent light detection plane, as illustrated in

the drawings, is image-formed as a 1212 point onto an

end of one optical fiber in an optical fiber bundle 322

by a fluorescent light detecting microlens array 321,

15   then being launched into the one optical fiber. The

one optical fiber is formed of a core 3221 through

which a light passes and a portion 3222 for protecting

the core. The diameter of the core through which the

light passes is somewhat larger than that of the

20   (multi) fluorescent light point 1211 on the fluorescent

light detection plane Σ_{PH}. If, however, the wavelength

separating beam splitter 30 performs no rotation, the

1211 point is displaced to 1212, 1213, 1214 ⋯ ⋯ (FIG.

6E) by the driving of the AO deflector 12, then being

25   shifted from the optical fiber light-receiving end.

This makes the detection impossible. Accordingly, as

illustrated in FIG. 7, using the piezo device 301

having the high resonant frequency characteristic, the

wavelength separating beam splitter 30 is driven so
that it performs the microscopic rotation.

Namely, in FIGS. 6A to 6E in the case of no
microscopic rotation, the fluorescent light microscopic
5   spot image 1211 is displaced to the different locations
1212, 1213, 1214. On the other hand, as illustrated
within a dotted-line frame in FIG. 7, driving the beam
splitter to perform the microscopic rotation permits
the spot to come onto substantially the same position
10   on the plane Σ_{PH}.

FIGS. 8A to 8F illustrate the relative time
variations among the following respective signals: S_{A12},
an ON-OFF signal or an intensity modulation signal of
the above-described AO deflector 12, S_{B12}, the deflec-
15   tion signal of the AO deflector 12, S_{30}, a driving
signal of the piezo device 301 for deflection-driving
the wavelength separating beam splitter 30, S_{33K}, a
fluorescent light detection signal detected by one k-th
photomultiplier tube 33 through a fiber 32, S'_{33K}, a
20   per-pixel image storage (integration) signal of the
fluorescent light detection signal,   and S''_{33K}, a per-
pixel final result of this image storage (a value
obtained by a sampling-and-holding of S'_{33K} at a point-
in-time before the image storing has been performed and
25   the multi-spot excitation lights transfer to the
excitation of the next pixel). In the embodiment of
this graph, the multi-spot excitation lights are
shifted in sequence on the one-pixel basis by the AO

deflector 12, and in this way, one spot has been shifted in sequence by the amount of ten pixels.

In order to enhance a signal-to-noise ratio in the detection of each pixel, the number of this

5 shifting need to be equal to 2 or more. The larger the number is, the more desirable it is to implement the enhancement of the signal-to-noise ratio. However, conditions such as restrictions on the apparatus's configuration components naturally impose an upper

10 limit on the number. Nevertheless, making the number equal to 5 or more outstandingly reduces the influences of the scattered lights or the fluorescent lights accompanied by the foreign substance irradiation with the adjacent multi-spot excitation lights halfway in

15 the optical paths.

As illustrated in FIGS. 8A to 8F, the frequency (the frequency of an ultrasound applied to an ultrasound quartz oscillator, i.e., the deflection angle of the deflector) signal S_{B12} of the AO deflector

20 12 is changed in sequence in a step-like manner. During this change, the driving signal S_{30} of the piezo device 301 for deflection-driving the wavelength separating beam splitter 30 is changed linearly in response to this change. The AO deflector 12 diffracts

25 a light by an extremely slight amount of change of a transparent medium's refractive index caused by the ultrasound propagation. Meanwhile, the piezo device 301 drives the beam splitter 30 as a whole. As a

result, there exists a difference in the frequency
response characteristic between them. Consequently,
the fast frequency signal S_{B12} of the AO deflector 12 is
formed into the step-like configuration and the slow

5   driving signal S_{30} of the piezo device is formed into
the linear configuration.

FIG. 9 is a diagram for illustrating the
positions of the fluorescent light spot images formed
by these two signals on the fluorescent light detection

10   plane Σ_{PH}. The change from the above to the below in
this drawing represents the lapse of a time t. Two
graphs on the left side indicate the changes of S_{B12} and
S_{30}. Although the number of the steps is equal to 10 in
FIG. 8, it is equal to 5 in this drawing. Solid-line

15   circles 3211', 3221', 3231', ⋯ ⋯ that exist on the
right side of the graph in FIG. 9 indicate the position
shift of the fluorescent light detection multi-spot
image in the case where, as was explained in FIGS. 6A
to 6E, the position correction by the piezo device has

20   not been performed yet. Incidentally, the right direc-
tion (transverse direction) in the drawing represents
the arrangement direction x_A of the spot array, and
solid-line circles 3212', 3222', 3232', ⋯ ⋯ indicate
the position shift of the fluorescent light spot image

25   adjacent to the above-described image. Moreover,
dotted-line circles indicate the consequences obtained
as the result of executing the position correction by
the piezo device.

Namely, even when the AO deflector 12 had

finished performing the step displacement of the

excitation light and thus the excitation light has

already stopped, the piezo device is still driving the

5 deflection linearly. As a result, the corrected

position of the fluorescent light spot image formed on

the fluorescent light detection plane Σ_{PH} is displaced

as is indicated by 3211_-, 3211, 3211_+ by a slight amount

though, while the excitation light continues to stop

10 (The corrected position of the adjacent fluorescent

light spot image is displaced as is indicated by 3212_-,

3212, 3212_+ by a slight amount, though).

When the excitation light has been step-

displaced to the next irradiation position on the DNA

15 chip, and even if it continues to stop there, the

corrected position of the fluorescent light spot image

is displaced as is indicated by 3221_-, 3221, 3221_+ by a

slight amount, though (The corrected position of the

adjacent fluorescent light spot image is displaced as

20 is indicated by 3222_-, 3222, 3222_+ by just a slight

amount, though). In order to cover and detect this

slight amount of displacement of the fluorescent light

spot images, as illustrated in FIG. 10, there is

provided an arrangement of ellipse-shaped apertures

25 3201, 3202, $\cdots\cdots$, $320M$ on a fluorescent light detection

light-receiving plane (or, a plane conjugated with the

light-receiving plane) 320, the longer radius of the

ellipse being in the spot array direction x_A. In the

case of the above-described detection using the fiber,
it is all right to set a condition that the fiber
light-receiving end plane should include the ellipse.

FIG. 11 is a diagram for illustrating an
5   embodiment in the present invention. The same refer-
ence numeral as that in FIG. 1 denotes the same
component or the same function. In the present
drawing, there is illustrated only a portion relating
to the generation of the multi-spot excitation lights
10 out of the entire apparatus in FIG. 1. The configura-
tion components other than the portion are basically
the same as those in FIG. 1. A reference numeral 14'
denotes a multi-spot generating hologram.

FIG. 12 is a diagram for illustrating a
15 method of creating the multi-spot generating hologram.
As illustrated in FIG. 12, a laser light is irradiated
with a mask 18 having pinhole array $1j_11'$, $1j_12'$, $\cdots \cdots$,
$1j_1M'$ apertures having hole diameters corresponding to
dimensions of the multi-spot excitation lights. Then,
20 the transmittance lights having passed through the mask
are converged onto a hologram storage medium by a
Fourier transformation lens 15. Next, the converged
position is irradiated with a reference light 1000 from
a somewhat oblique direction at an incident angle $\phi = \phi_0$
25 in a superimposed manner, thereby forming the Fourier
transformation hologram at the above-described
converged position.

In order to enhance the light utilization

efficiency, there is employed a phase-modulation type storage medium. Also, random phases that are independent of the mutual positions of the respective apertures are added to the apertures. This addition is performed

5 in order to avoid the following situation: The intensity of a 0th light at the central position (a position where the space frequency is equal to 0) on the Fourier transformation surface is increased up to an order-of-magnitude difference, and thus values such

10 as the signal-to-noise ratio and the diffraction efficiency of the formed hologram become worse.

The hologram created in this way is used in the multi-spot excitation lights-generating system in the DNA inspecting apparatus in FIG. 11.

15 In FIG. 11, a laser light is launched into the AO deflector 12 and then passes through the lens system 13. Next, the hologram 14' that has been created by the above-described method is irradiated with the laser light at an incident angle ϕ_0. An

20 incident angle ϕ of the laser light launched into the hologram is set so that, at the center (the center of the deflection angle) of the driving frequency of the AO deflector 12, the incident angle ϕ becomes equal to the incident angle ϕ_0 of the reference light 1000

25 toward the hologram surface at the time of creating the hologram by the method in FIG. 12. The irradiation of the hologram with the laser light in accordance with this setting, as illustrated in FIG. 11, reproduces, at

$1j_11$, $1j_12$, ⋯ ⋯, $1j_1M$, a spot array that is the same as the pinhole array at the time of creating the hologram in FIG. 12.

   Next, changing the frequency of the AO
5 deflector brings about a slight amount of change in the incident angle into the hologram, thereby bringing about a slight amount of change in the angles of the multi-spot reproduced lights as well. As a result, it turns out that a position equivalent to the adjacent
10 pixel is illuminated and excited. Accordingly, a sequential driving of the AO deflector makes it possible to execute the multi-spot excitation illumination of the DNA chip in the step-like manner, i.e., in such a manner that the position to be irradiated is
15 changed in sequence on the one-pixel pitch basis.

   FIG. 13 is a diagram for illustrating another embodiment of the DNA inspecting apparatus in the present invention. The same reference numeral as that in FIG. 1 denotes the same component. In the present
20 embodiment, unlike the embodiment in FIG. 1, the detection in a 2-dimensional manner is executed as well simultaneously. Namely, the excitation illumination light is caused to pass through an illuminating lens 13′ capable of simultaneously illuminating a 2-
25 dimensionally-broad range. Moreover, the excitation illumination light is reflected by a wavelength selection beam splitter 30′, then illuminating a 2-dimensional microlens array 14′. The 2-dimensional

microlens array 14' has the same functions as those in the case where the 1-dimensional microlens array has been already explained using FIGS. 1 to 4, and what differs therefrom is the 2-dimensional array arrange-

5 ment alone. Consequently, as illustrated in FIG. 14, 2-dimensional microscopic spots 1410' are formed at focal point positions of microlenses 141'.

In the case of the present embodiment, pin-hole apertures are bored with the positions of 1410' as

10 their center. In this way, the DNA chip is irradiated with only the lights that have passed through the pinhole apertures. A ratio between a diameter d of the microscopic multi spots and an arrangement pitch p of the spots is equal to an integer that is larger than 2

15 and that, preferably, is larger than 5. As illustrated in FIG. 13, a high-resolution lens 16' causes the DNA chip to be simultaneously irradiated and excited with the 2-dimensional multi-spot lights having passed through the pinhole apertures. The pixels undergoing

20 the excitation irradiation, i.e., $2x_{11}y_{11}$, $2x_{12}y_{11}$, $2x_{11}y_{21}$, $2x_{21}y_{11}$, $\cdots$ $\cdots$ have an equal pitch spacing with 4 pixels skipped.

Fluorescent lights generated from the above-described pixels that have simultaneously undergone the

25 excitation irradiation pass through the above-described high-resolution lens 16', then passing through the respective pinholes 1410' on the lower surface of the microlens array. The fluorescent lights having passed

through the pinholes pass through the respective micro-
lenses, thereby being enlarged up to a size of the

upper surfaces (convex surfaces) of the microlenses.

The fluorescent light intensities on the upper surfaces

5   of the microlenses are image-formed onto a high-

sensitivity image storage type 2-dimensional sensor 32'

through the wavelength selection beam splitter 30' and

an image-forming lens 31'. Although the interference

filter is not illustrated in the present drawing, the

10 interference filter, or the color filter that permits a

wavelength longer than the fluorescent lights to pass

through is provided between the wavelength separating

beam splitter 30' and the 2-dimensional sensor 32'.

In the 1-dimensional and 2-dimensional

15 microlens arrays illustrated in FIG. 4 and FIG. 14,

respectively, a light incoming into an intermediate

area between the adjacent microlenses in the respective

microlenses is in a danger of becoming a scattered

light and then a noise light. Thus, by performing a

20 masking (not illustrated) by which the intermediate

area is covered with a light-shielding portion composed

of a material such as chromium oxide, it becomes

possible to eliminate such a noise.

FIG. 15 and FIG. 16 illustrate a spectro-

25 scopic reflection characteristic and a spectroscopic

transmittance characteristic respectively of the wave-

length separating beam splitters 30, 30' and the

interference filter 34, which have been used in the

above-described embodiments and in FIGS. 1, 5, 7, and
13. The use of both of the apparatuses makes it
possible to decrease the influences of the excitation
lights, thereby allowing the precise detection to be
5 executed. In the drawing, λ denotes a wavelength of
the excitation lights. The excitation wavelength
bandwidth is narrow because the laser light is usually
used in order to increase a per-unit area intensity of
the spot irradiation. λ_L denotes a central wavelength
10 of the fluorescent lights to be detected.

As the fluorescent material added to the DNA
fragment to be hybridized, several types of fluorescent
substances are employed. For example, when employing
often employed Cy5 (Cyanine5), an absorption peak
15 wavelength of the fluorescent material is equal to 649
nm and a peak wavelength of the fluorescent lights is
equal to 670 nm. Also, on an even shorter wavelength
side, when employing Cy3 (Cyanine3), the absorption
peak wavelength of the fluorescent material is equal to
20 550 nm and the peak wavelength of the fluorescent
lights is equal to 570 nm. Since the spectroscopic
absorption characteristic of the absorbing material has
a bandwidth, it is not always required to cause the
wavelength of the excitation laser light to coincide
25 with the absorption peak wavelength. Accordingly,
there is used a laser light the wavelength of which is
close to the absorption peak wavelength.

In the case of Cy5, there is used a red He-Ne

laser light at the wavelength of 633 nm or a semicon-
ductor laser light at the wavelength of 635 nm. In the
case of Cy3, there is used a green He-Ne laser light at
the wavelength of 544 nm or the like. In the inter-

5 ference filter and the wavelength separating beam
splitter for extracting only the fluorescent lights, as
the central wavelength, a wavelength is selected which
is close to the fluorescent light peak wavelength and
at which it is easy to separate the excitation lights.

10 In the above-described fluorescent light
detection, it becomes of the utmost importance to
completely light-shield the excitation lights especial-
ly when the fluorescent lights are weak.

FIG. 17 is an embodiment-illustrating diagram

15 in the present invention, where it is intended to
execute the above-described light-shielding of the
excitation light more completely. Namely, the light-
shielding based on the present embodiment is executed
in the following cases: The use of the interference

20 filter or the wavelength separating beam splitter alone
is insufficient for the light-shielding, or it is
desirable to increase the band half-value width of the
interference filter in order to increase the fluo-
rescent light detection intensities. In FIG. 17, the

25 same reference numeral as that in FIG. 1 have the same
component or the same function.

A multi-spot array 111, ⋯ ⋯, 11M formed on a
plane Σ_f by the microlens array or the hologram is

image-formed onto the DNA chip as excitation lights
through the operation of the lens 15 and the objective
lens 16. At this time, the respective multi-spot
lights pass through a center of an incident pupil EP_0 of
5   the objective lens with a spread the radius of which is
substantially RNA_σ. Setting a numerical aperture and
the focal length of the objective lens to be NA and f,
respectively, a spot diameter Ds of the excitation
lights on the DNA chip is given by the following
10   equation:

$$Ds = 2k_1 \ f\lambda \ / \ RNA_\sigma' \qquad (where \ k_1 = 0.6)$$

Representing, by p, a pixel resolution (the pixel
pitch) for the DNA chip detection, this value is
substantially equal to Ds. Assuming that p is equal to
15   2 μm and the excitation light wavelength λ is equal to
633 nm, a value RNA_σ'/f becomes equal to 0.19. The
value RNA_σ'/f is a NA of an illumination for illuminat-
ing the spot of 2 μm (i.e., a minimum NA of the objec-
tive lens needed in order to image-form the 2 μm spot).
20   Representing this value by NA', the NA' is given as
follows:

$$NA' = sin(tan\text{-}1 \ (RNA_\sigma' \ / \ f)) \fallingdotseq RNA_\sigma' \ / \ f = 0.19$$

In order to detect the weak fluorescent lights, the NA
of the objective lens 16 falls in the range of 0.7 to

0.9.

Next, the respective excitation spot lights,
which have passed through the incident pupil of the
objective lens with the above-described spot diameter,
5   irradiate the DNA chip with the excitation spot lights
of about 2 μm through the operation of the objective
lens. Since the objective lens is both-telecentric in
nature, about 4 to 8% of the excitation lights out of
the excitation lights incoming into the DNA chip in
10   perpendicular thereto are regularly reflected by the
chip surface, then returning back to the objective
lens. The wavelength separating beam splitter permits
most of the regularly reflected excitation lights to
pass through, but the beam splitter reflects the
15   regularly reflected excitation lights by just a slight
amount. Then, the slight amount of reflected excita-
tion lights travel toward the fluorescent light detect-
ing system 3.

The fluorescent light detecting system 3
20   includes the interference filter 34, thereby light-
shielding the excitation lights. Nevertheless, the
complete light-shielding is a difficult task. Namely,
trying to widen the bandwidth of the interference
filter so as to detect the fluorescent lights as much
25   as possible results in just a slight amount of leakage
of the excitation lights. Thus, a lens system 31' for
image-forming and detecting the fluorescent spot lights
onto a fluorescent light detection plane (or, a plane

conjugated therewith) is configured with lens systems
311, 312 as illustrated in the drawing. This configuration is designed so as to image-form the objective
lens pupil at a position existing between 311 and 312.

5 A space filter 35 is located at this position
conjugated with the pupil. The space filter 35 has a
configuration as illustrated in FIG. 18.

The light-forming magnification of the
objective lens pupil' surface toward the space filter

10 35 by the lens system 311 is assumed to be 1 time. The
space filter has a circle-shaped light-shielding
portion 351 of a radius RNA_σ in the central portion of
an aperture, the aperture having a radius RNA that is
equal to a radius of the objective lens pupil from the

15 optical axis center of a light-shielding portion 353.
The multi-spot excitation lights, which had been
regularly reflected by the DNA chip, turns out to have
the beam radius of $RNA_{\sigma'}$ on the space filter.
Consequently, if $RNA_\sigma > RNA_{\sigma'}$ holds, the regularly

20 reflected excitation lights are light-shielded by the
light-shielding portion 351.

Meanwhile, the fluorescent lights excited and
generated by the multi-spot excitation lights are
launched into the objective lens with almost no direc-

25 tivity, then incoming into the space filter. The
light-shielding portion 351 light-shields the fluo-
rescent lights as well, but a ratio of the light-
shielded fluorescent lights toward the space filter

incoming fluorescent lights becomes equal to $(NA'/NA)^2$.
Substituting the above-described value, this value is
found to be 7 (when NA = 0.7) to 4 (when NA = 0.9) %,
which is a loss of a negligible order.

5   In this way, it is possible to reduce the
unnecessary excitation lights tremendously without
lowering the light amount of the fluorescent lights to
be detected. The fluorescent light detection lights
having passed through the space filter pass through the
10   lens 34 and the interference filter 34, then image-
forming the spot images on the DNA chip onto the detec-
tion plane, i.e., the optical fiber light-emitting
ends, so as to be subjected to the fluorescent light
detection.

15   FIG. 19 is an embodiment-illustrating diagram
in the present invention, which indicates a method of
preventing the excitation lights from being guided into
a fluorescent light detecting optical path. The same
reference numeral as that in FIG. 1 denotes the same
20   component. In the present embodiment, a polarization
beam splitter 30" is employed as a beam splitter for
branching off the fluorescent light detecting optical
path from an excitation light optical path. Namely, a
split surface of the polarization beam splitter is
25   irradiated with the excitation lights that are P-
polarized toward the split surface. Then, since the
excitation lights having been reflected on the DNA chip
surface and having returned back still remain P-

polarized, the excitation lights pass through the polarization beam splitter and never enter the fluorescent light detecting optical path. On the other hand, since the polarization state of the generated

5   fluorescent lights differs from that of the excitation lights, the S-polarized fluorescent lights are reflected by the polarization beam splitter 30", then being guided into the fluorescent light detecting optical path. In this way, this method makes it

10 possible to prevent the excitation lights from being guided into the fluorescent light detecting optical path.

FIG. 20 is an embodiment-illustrating diagram in the present invention, where the fluorescent light

15 detection is executed using the optical fibers and the multi-channel photomultiplier tube. Namely, FIG. 20 illustrates the concrete content of the detection of the fluorescent light multi-spot images in the embodiments illustrated in FIGS. 1, 5, 7, 17, and 19. As

20 illustrated in FIG. 21, the light-receiving end of the fiber system 32 for the fluorescent light multi-spot images the multi-spot number of which is M or more is the multi-lens array 321 including 1-dimensionally arranged M lenses.

25   As having been explained in FIG. 7, using the respective microlenses, the fluorescent light multi-spot images are launched into the cores through which a light passes in the fibers. If, as illustrated in FIG.

20 the multi-channel photomultiplier tube 33 includes

2-dimensionally arranged light-receiving apertures, the

light-emitting ends of the fibers are also configured

to be 2-dimensionally arranged. In the case of the

5 embodiment in FIG. 20, the fluorescent lights emitted

from the respective light-emitting ends are image-

formed by the image-forming lens 324 in such a manner

that the fiber light-emitting ends 323 correspond to

the respective light-receiving apertures 331 of the

10 photomultiplier tube 33.

In the case of the embodiment in FIG. 21, a

2-dimensional lens array 3241 is provided at the light-

emitting ends of the fibers in the corresponding

manner. The fluorescent lights emitted from the

15 respective fibers pass through the respective lenses,

thereby being directly converged onto 2-dimensional

light-receiving apertures (photoelectric plane) 3311 of

the multi-channel photomultiplier tube. In the embodi-

ments illustrated in FIGS. 20, 21, the 2-dimensional

20 multi-channel photomultiplier tube is employed. When

using the 1-dimensional multi-channel photomultiplier

tube as well, configuring the light-emitting ends to be

1-dimensionally arranged also allows the detection to

be executed by the same method.

25 FIG. 22 is an embodiment-illustrating diagram

in the present invention. In the present embodiment,

as the excitation lights, there are used excitation

lights having a plurality of different wavelengths.

Light-source systems 11A, 11B, and 11 C form lights emitted from excitation light light-sources with different wavelengths λ_A, λ_B, and λ_C, then causing the lights to be launched into fiber bundles. In the

5 light-source systems, multi-lens arrays are arranged at the light-emitting ends, and pinhole arrays are provided at the positions at which the lights after being emitted are substantially converged, and the excitation lights having been emitted from the pinhole

10 arrays are configured so that the excitation lights pass through wavelength separating beam splitters 30A, 30B, and 30 C. As illustrated in FIG. 22, when the system 11A is selected as the excitation light light-source system for the fluorescent light detection, the

15 excitation light with λ_A passes through the lens 15, and is reflected by a deflection mirror 300, then passing through the objective lens 16 so as to perform multi-spot excitation irradiation toward the DNA chip 2. Moreover, fluorescent lights generated from the

20 respective multi spots pass through the objective lens 16, the deflection mirror 300, and the lens 15 (31). Then, the fluorescent lights are reflected by the wavelength separating beam splitter 30A, eventually passing through an interference filter 34A so as to be

25 detected by the method explained in FIGS. 20, 21 using the multi-channel photomultiplier tube.

When the fluorescent lights to be detected are of different types, the entire light-source system

50 (i.e., 11A, B, C; 120A, B, C; 130A, B, C; 30A, B, C; 34A, B, C) is displaced in solid-line arrow directions in accordance with a control apparatus 4' by a driving mechanism not illustrated. Then, either of the

5   different wavelengths λ_B and λ_C is selected so as to execute the fluorescent light detection using this selected wavelength. When the plurality of types of fluorescent lights are to be used for one DNA chip, the light-source systems are displaced in sequence, thereby

10 executing the detection using the different excitation lights one after another.

FIG. 26 is another embodiment-illustrating diagram, where the above-described plurality of types of fluorescent lights are used. Unlike the case in

15 FIG. 22, irradiations with the plurality of types of excitation lights are performed simultaneously, thereby trying to shorten the detecting time. In FIG. 26, excitation lights, which are emitted from light-source systems 11A', 11B', and 11 C' with a plurality of

20 different wavelengths λ_A', λ_B', and λ_C' corresponding to, for example, 3 colors of red, green, and blue, are synthesized effectively into one optical path by wave-length selection synthesizing mirrors 51, 52. Namely, the wavelength selection synthesizing mirror 51 permits

25 blue to pass through and causes green to be reflected. Also, the wavelength selection synthesizing mirror 52 permits red to pass through and causes green and blue to be reflected.

The synthesized 3 colors pass through a beam splitter 30' and, after passing through the lens 15, the deflection mirror 300, and the objective lens 16, perform 3-color simultaneous spot excitation illumina-

5 tions with the 3 colors onto the DNA chip. The respective fluorescent materials are excited at each wavelength and emit lights with the respective fluorescent colors. The fluorescent lights emitted have wavelengths that are slightly longer than those of the

10 excitation lights. This condition makes it possible to separate, using wavelength separating beam splitters 53, 54, the wavelengths of the 3-wavelength fluorescent lights excited by the 3-wavelength excitation lights. Namely, the wavelength separating beam splitter 53

15 causes the blue fluorescent light to be reflected and permits the green and the red to pass through. Also, the wavelength separating beam splitter 54 causes the green to be reflected and permits the red to pass through.

20 In optical paths resulting from the separation into the respective 3 colors in this way, there are located interference filters 34C, 34B and the filter 34A that each permit the respective fluorescent lights alone to pass through with a high-purity. Then,

25 the microscopic spot images of the respective fluorescent lights are detected simultaneously by the above-described method using multi-channel photo-multiplier tubes 33C', 33B', and 33A' through fibers.

In the embodiments in FIGS. 22, 26, the
deflection mirror 300 performs the array direction
scanning of the excitation multi-spot lights. As the
deflection mirror, there is employed a mirror of the
5 piezo driving system or of the galvanometer mirror
type. In this case, unlike the embodiment explained in
FIG. 1, the multi spots are subjected not to the step
displacement but to a continuous (linear) scanning.
The multi spots on the DNA chip are scanned, but one
10 and the same (identical) deflection mirror is used in
the detecting optical path as well. As a result,
unmoved multi spots are image-formed at the ends of the
fluorescent light detecting fibers. On account of
this, it turns out that pixel addresses are determined
15 based on the deflection angle of the deflection mirror.

Based on the relationship between the pixel
addresses and the deflection angle, a plurality of
fluorescent light detection signals obtained from the
multi-channel photomultiplier tubes in a parallel
20 manner are organized and stored by the control
apparatuses 4' (FIG. 22) or 4" (FIG. 26). Such data
processing, of course, requires that the measuring and
detecting conditions be inputted in advance. These
pieces of input information are inputted from a
25 terminal 41' or from a high-order computer 40then, as
necessary, data on the measured and inspected result is
transferred to the computer.

In the embodiment in FIG. 26, the explanation

has been given concerning the case where the irradia-
tions with the plurality of excitation lights having
the different wavelengths are performed simultaneously.
By the way, in the case where there exist an overlap

5 between the wavelength bands of the plurality of
excitation lights and those of the fluorescent lights
at which the respective excitation lights aim, a
shutter situated near the light-sources and not
illustrated is used toward the overlapped excitation

10 light or the fluorescent light detection is executed
with the time shifted by an ON-OFF of the light-sources
themselves.

FIG. 23 is an embodiment-illustrating diagram
in the present invention, where a plurality of semicon-

15 ductor lasers 111A2, 111A2, ··· ··· having substantially
the same wavelength are employed as the excitation
light light-source. The semiconductor laser is of a
small volume, producing a comparatively high-output,
and is inexpensive. As a consequence, as illustrated

20 in the drawing, configuring a multi-spot light-source
using a large number of semiconductor lasers makes it
possible to irradiate the DNA chip with intense multi-
spot excitation lights, thereby allowing the high-speed
detection to be implemented. The lights emitted from

25 the respective semiconductor lasers are taken into
light-receiving ends of fibers 120A1, 120A2, ··· ··· by
lenses 112A1, 112A2, ··· ····.

The laser lights emitted from the light-

emitting ends, as illustrated in FIG. 24, are converged

onto a pinhole arrangement 111, 112, ⋯ ⋯ through a

microlens array. Then, the transmittance lights having

passed through the pinhole arrangement are used as the

5   multi-spot excitation lights.

     If the semiconductor lasers are not usable as

the excitation lights, there are used high-output

solid-state lasers excited with the semiconductor

lasers, and high-output gaseous lasers. In such laser

10   light-sources, by using an emitted beam in such a

manner as to be split by a method illustrated in FIG.

25, it becomes possible to form multi-spot excitation

lights having substantially the same intensity and

substantially the same beam configuration.

15   Namely, the beam emitted from a laser light-

source 111A' is split using multi-splitting beam

splitters 1110A', 1120A', ⋯ ⋯. It is assumed that the

number of the splitting is equal to k and reflectances

of beam-split lights from the 1st stage of the split-

20   ting through the 2nd stage, the 3rd stage, ⋯ ⋯ to the

k-th stage are equal to r_1, r_2, ⋯ r_j, ⋯ r_k. Considering

that r_k is equal to 1 and since the respective beam-

split lights are equal in their intensities, with

reference to an arbitrary j ($1 \leqq j \leqq k$),

25        $r_j = 1 / (k - j + 1)$

should be satisfied in order to form the above-

described multi-spot excitation lights. Namely, at
1110A′, since k is equal to 4, i.e., 4-splitting, r_1,
r_2, r_3, and r_4 become equal to 1/4, 1/3, 1/2, and 1,
respectively. Similarly, since 3-splitting is

5   performed at 1120A′, r_1, r_2, and r_3 become equal to 1/3,
1/2, and 1, respectively. In this way, the laser
lights having the same intensity and the same beam
configuration are launched into a lens array 112A1′,
then being launched into cores of the fibers.

10   In the embodiment in FIG. 23, the plurality
of semiconductor lasers are employed as the excitation
light light-source for the generation of the multi-beam
spots. In the case where the available laser light
power is inadequate, however, it becomes required to

15   form the multi-spot excitation lights with the use of a
plurality of gaseous lasers other than the semicon-
ductor lasers or a plurality of lasers based on the 2nd
harmonics. In such a case, the systems illustrated in
FIG. 25 are used in plural number and the fiber light-

20   emitting ends picked up from the plurality of systems
are arranged on a 1-dimensional line or a 2-dimensional
plane. This makes it possible to obtain the multi-spot
excitation lights with a high-excitation intensity.
          In the above-described case where the

25   plurality of excitation lights are used, depending on
the excitation wavelength, there arises a necessity of
using various types of different lasers, such as the
semiconductor laser, the gaseous laser, and the solid-

state laser using the 2nd harmonics excited with the semiconductor. In such a case, it is all right to form the multi-spot excitation lights in accordance with the above-described method illustrated in FIG. 23 or FIG.

5   25.

FIGS. 27, 28 are embodiment-illustrating diagrams in the present invention. In the drawings, by eliminating the influences of the fluorescent lights and the scattered lights generated by the excitation

10 multi-spot lights from a foreign substance mixed in the DNA chip, or the influence of a scattered light from a foreign substance in the fluorescent light detecting optical system, it is made possible to implement the high-accuracy fluorescent light detection. A reference

15 numeral 11' denotes an excitation light-source, which causes a light to pass through a pinhole aperture array 14' through a fiber so as to create the excitation multi-spot lights. The multi-spot lights are image-formed onto the DNA chip through the operation of the

20 lens 15 and the objective lens 16. A space filter 161 is located at the position of an incident pupil of the objective lens.

The excitation multi-spot lights are set so that the multi-spot lights pass through a position 163

25 (refer to FIG. 28) shifted off from a center of the incident pupil. Namely, as illustrated in FIGS. 29, 30, the respective multi-spot lights pass through a portion shifted off from an optical axis of the

objective lens, becoming a light-flux Ii represented by an oblique sloped line in FIG. 30. Then, the DNA chip 2 is irradiated with this light-flux in an oblique direction at an incident angle θi. As having been

5 explained earlier in the relationship between the spot diameter and the NA', a convergence angle θI of this irradiation converging light is expressed by (NA') = $\theta I/2$.

Also, the excitation lights reflected

10 regularly by the DNA chip pass through the objective lens, then passing through a position (the position indicated by 162 in the drawing) that is symmetrical with 163 with the lens' optical axis as the symmetrical center on the pupil 161 (refer to FIG. 28).

15 Thus, as illustrated in FIG. 28, forming, in this position, a member 162 for light-shielding the regularly reflected excitation lights allows the regularly reflected excitation lights to be light-shielded by this member 162. In this way, the position

20 off from the center of the pupil is set to be the optical path of a primary light-ray of the excitation lights. Moreover, with reference to the incident angle θi of this primary light-ray, a condition $\theta i > \theta I/2$ is made to be satisfied. This condition makes it

25 possible to light-shield the regularly reflected excitation lights on the pupil without light-shielding the excitation lights on the pupil. It is needless to say that the present embodiment has the effect of

eliminating the noise caused by the excitation lights, which has been explained in the embodiment in FIG. 17.

In the embodiment in FIG. 27, in addition to this effect, the influences of the foreign substance

5 mixed on or in the DNA chip are eliminated in the following way: Namely, if there exists the foreign substance such as various kinds of proteins mixed at the time of forming the DNA sample, the irradiation of the foreign substance with the excitation lights causes

10 the excitation lights to be scattered, because the dimension of such a foreign substance is small, i.e., several μm. Also, in the case of the above-described foreign substance of the organic substance type, intense fluorescent lights are emitted from the foreign

15 substance, becoming the fluorescent lights that are more intense than the fluorescent material added to the DNA to be detected.

As illustrated in FIG. 30, the scattered excitation lights pass through a dotted-line surrounded

20 area L + S determined by the NA (= $\sin\theta_{L+S}$) of the objective lens. Then, after passing through the objective lens, the scattered excitation lights pass through a portion other than the light-shielding member 162 of the space filter, leaking into the fluorescent

25 light detecting system. Only this leakage of the scattered excitation lights is extracted using a wave-length separating beam splitter 61, then executing the image detection. The wavelength separating beam

splitter permits the fluorescent lights to pass through
and causes the excitation lights to be reflected.
Although, in FIG. 29, the wavelength separating beam
splitter 61 is inserted in optical paths behind the

5 wavelength separating beam splitter 30″, it may be
inserted in optical paths before the beam splitter 30″.
In this way, in much the same manner that the fluo-
rescent light image is photographed by a detector 33′
such as the multi-channel photomultiplier tube, an

10 excitation light scattered image S100 ((refer to FIG.
29.) of the excitation lights scattered by the foreign
substance is detected as an image by a detector 62.

FIGS. 31A, 31B indicate, at a signal level on
the per-pixel basis, a fluorescent light image signal D_L

15 and an excitation light image signal D_s that have been
detected in this way. Namely, numerals 1, 2, ⋯ ⋯, 25
denote detected pixel addresses and numerals (1), (2),
(3), (4), (5) denote addresses of cells to which the
same DNA sequence is hybridized. Namely, for example,

20 the pixels at the addresses 1, 2, 3, 4, 5 belong to the
cell (1). Although, actually, the pixels in the cells
are 2-dimensionally located and there exist, for
example, 5×5 pixels, the pixels are 1-dimensionally
located in order to make the drawings-accompanying

25 explanation easy to understand. If the detection is
exerted upon a pixel where the foreign substance
exists, the scattered excitation light from the pixel
exceeds a threshold value D_{ST}.

In FIG. 31B, at the pixel addresses 9, 10, 11, 12, and 16, 17, 18, 19, 20, the excitation light image signal D_S exceeds the threshold value D_{ST}. Consequently, the information on the fluorescent light

5 image signal D_L existing at the corresponding addresses is strongly influenced by the foreign substance. Accordingly, an average value of the fluorescent lights in each cell is determined by discarding the information at these addresses. Namely, concerning the cell

10 (2), the average value is determined not by using the information at the addresses 9, 10 but by using only the information at the remaining addresses 6, 7, 8. Similarly, concerning the cell (3), the average value is determined by using only the information at the

15 addresses 13, 14, 15. In the cell (4), the scattering from the foreign substance occurs in all the pixels, and thus this cell is regarded as being invalid. The average intensity in each cell is determined in this way as is illustrated below in the graph in FIG. 31C.

20 This allows the influences of the foreign substance to be reduced tremendously, thereby making it possible to execute the precise fluorescent light detection.

Incidentally, in the optical systems of the embodiments explained using FIGS. 27 to 30 where the

25 influences of the foreign substance are eliminated, there are provided the optical systems where the excitation lights illuminate the DNA chip after passing through the objective lens. However, the configuration

is also allowable where the irradiation with the excitation lights is performed from between the objective lens and the DNA chip in an oblique direction without passing through the objective lens. In this

5 case, since the regularly reflected excitation lights are not launched into the objective lens, a space filter (which is equivalent to 161) becomes unnecessary.

FIG. 32 is an embodiment-illustrating diagram

10 in the present invention. The reference numerals denote the following components: 1 the multi-spot excitation lights-irradiating system the detailed embodiments of which have been explained already, 3 the fluorescent light detecting system for detecting

15 the fluorescent lights generated by the multi-spot excitation lights, the details of which have been also explained already. The fluorescent material attached to the DNA hybridized onto the DNA chip 2 is irradiated with the microscopic multi-spot excitation lights of

20 several μm through the use of the objective lens 16. In order to accomplish this, it is required to always maintain the distance between the objective lens and the fluorescent light plane at a fixed spacing within the focal depth so that the spot size is kept at a

25 constant value. For the purpose of this, there is used a focal point detecting system 7 that is structurally integrated with the objective lens 16 for implementing the rigidity. The focal point detecting system 7

includes an oblique beam spot irradiating system 71 and a spot position detecting system 72. The oblique beam spot irradiating system 71 irradiates the fluorescent light plane on the DNA chip with the microscopic spots

5 from an oblique direction.

The irradiation with the spots is performed, and the spacing between the objective lens 16 and the fluorescent light plane is detected. Then, the objective lens 16 is controlled so as to be driven in the z

10 direction in accordance with the detection signal by an objective lens up-and-down driving mechanism 73 so that, even if a stage 80 for mounting the DNA chip 2 thereon is driven in x, y directions by a driving motor 83(84), the above-described fixed spacing is main-

15 tained. A numeral 82 denotes a length-measuring device of x, y stage.

FIG. 33 illustrates an example of a cross sectional structure of the DNA chip. In this embodiment-illustrating diagram, the fluorescent light

20 plane is a plane Σ_2. Namely, there exists the fluorescent light plane Σ_2 on a flat substrate 23 such as a glass. Between this plane and a plane Σ_1 on a glass substrate 21, there exists a clearance 22 into which a liquid containing the fluorescent material-added DNA to

25 be inspected is poured for implementing the hybridization. At the stage of the DNA inspection, the clearance is filled with the liquid. However, depending on the cases, the clearance is left empty. Also, in some

cases, the plane Σ_1 is selected as the fluorescent

light plane, and the substrate 23 is composed of an

opaque substance or a light-absorbing material.

A light beam f_0 irradiation of which is

5 performed from an oblique direction by the oblique beam

spot irradiating system 71 so that the light beam is

converged onto the fluorescent light plane, is

regularly reflected on the following 4 planes: An

upper plane Σ_3 and the lower plane Σ_1 of the upper

10 substrate 21, and the upper plane Σ_2 and a lower plane Σ_4

of the lower substrate 23. Light-rays l_3, l_1, l_2, and

l_4, which are formed by the regular reflections of a

primary light-ray f_{0M} of the oblique irradiation beam f_0,

are each image-formed by an image-forming lens 721 at

15 positions P_3, P_1, P_2, and P_4 on a plane equivalent to a

light-receiving plane of a light-receiving position

sensor 722. The position of the fluorescent light

plane is determined in advance and accordingly, if the

respective planes Σ_3, Σ_1, Σ_2, and Σ_4 are away from

20 each other to a certain extent, the following method

makes it possible to execute the height detection of a

desired detection plane alone: The dimension of the

light-receiving position sensor 722 and the image-

forming magnification of the lens 721 are determined

25 beforehand so that only a spot image resulting from the

reflected light-ray l_2 from the plane to be detected

(which is Σ_2 in this embodiment-illustrating diagram)

is received and the other spot images are image-formed

outside the light-receiving aperture.

Namely, in this embodiment, it becomes possible to capture only the regularly reflected light reflected from the plane Σ_2 and thus to detect the spot

5   position on the position sensor, i.e., the height position of the plane Σ_2. Then, based on this detected information and in accordance with the control apparatus 4, the objective lens and the focal point detecting system 7 integrated with the objective lens are

10   displaced slightly in an up-and-down direction by the driving mechanism 73. This allows the fluorescent light detection to be executed always in the focus-achieving state.

If the incident angle of the oblique inci-

15   dence focus detecting light in FIG. 33 is close to Brewster angle, causing the detecting light to be launched in the P-polarized state gives rise to an extremely small reflection on the plane surface, thereby making it difficult to execute the detection.

20   Accordingly, there is employed the S-polarized detecting light. The employment of the S-polarization enhance the reflectance on the plane surface more in comparison with that of the P-polarization no matter what value the incident angle is. This is advantageous

25   enough.

FIG. 34 is an embodiment-illustrating diagram in the present invention, where the focal point detection is executed through the objective lens 16. A

laser light having been guided by a fiber 74 from a near-infrared semiconductor laser light-source not illustrated, after being emitted from the fiber, passes through a beam splitter 76, a lens 77, and a wavelength

5 separating beam splitter 300". Then, the light passes through the objective lens 16 so as to be converged, and the fluorescent light plane on the DNA chip 2 is irradiated with the converged light from oblique directions. The incident angle θ of this irradiating

10 light is equivalent to the numerical aperture NA of the objective lens in its magnitude (an incident angle that is somewhat smaller than $\sin\theta$ = NA $\fallingdotseq$ 0.8). The regularly reflected light passes through the objective lens again, eventually forming, onto a position sensor

15 73', an image of the light-converged point on the DNA chip 2. Then, the position sensor 73' detects the position of this image. From this detected position, the height of the plane in the DNA chip can be deter- mined. The objective lens is displaced in the up-and-

20 down direction so that the image is image-formed at a reference point on the position sensor 73'. This, in the same way as the embodiment in FIG. 33, makes it possible to detect the focus position of the fluo- rescent light plane in the chip. Just like the present

25 embodiment, by employing the light the wavelength of which is longer than that of the detected fluorescent light as the light to be used for the focal point detection, it becomes possible to execute the focal

point detection precisely without exciting the fluo-
rescent material, i.e., without generating the detec-
tion noise.

Incidentally, the wavelength separating beam

5   splitter 300" in FIG. 34 permits the near-infrared
light to pass through and reflects the excitation light
and the fluorescent light used for the fluorescent
light detection. The irradiation with a 1-dimensional
excitation light spot array formed by a 1-dimensional

10   excitation light irradiating optical system 1 is
performed toward the fluorescent light plane in the DNA
chip through the lens 15, the wavelength separating
beam splitter 300", and the objective lens 16. A
fluorescent light detecting optical system 3 detects

15   the fluorescent light generated. The displacement of
the excitation light spot array irradiating positions
in the array direction is executed by causing the wave-
length separating beam splitter 300" to perform the
microscopic rotation.

20   Hereinafter, referring to FIGS. 35 to 40, the
explanation will be given concerning a method of
executing, all over the entire cells in the DNA chip,
the fluorescent light detection toward the DNA chip
that has been explained so far using the above-

25   described respective embodiments.

FIG. 35 illustrates the entire structure of
the DNA chip. A numeral 204 denotes the whole case for
housing the DNA chip. The DNA chip is a glass

substrate on an inner side of a window 200 formed on this case, and the chip is fixed to the case 204. The fluorescent material-added DNA fragment is hybridized with an area 202 on the inner side of the window. In

5   an area spreading outside the area 202 and inside the window 200, there are described alignment marks 201 for the positioning.

The relative positions between the cell 20 explained in FIG. 2 and the alignment marks 201 are

10   designed and formed with an accuracy of several-tenth μm, the cell 20 including N×N (in FIG. 5, 5×5) pixels (indicated by the bold line) in which the fragment of the same DNA information is planted (i.e., the probing is performed).

15   The DNA chip is mounted on the detecting apparatus the embodiments of which have been explained already. Then, in an alignment detecting optical system (not illustrated) implemented in the detecting apparatus, using a device such as a mark position

20   detecting 2-dimensional CCD, the positions of at least 2 of the alignment marks 201 are detected as positions on the CCD. Also, the position of the DNA chip at the time of executing this mark detection is detected using, for example as is illustrated in FIG. 32, the

25   length-measuring devices 81, 82 for detecting the position in the x and y directions. The length-measuring devices are provided on a chuck on which the DNA chip is mounted.

The spacing between the optical axis of the above-described CCD detecting optical system and the detecting optical axis in the previously-described various types of fluorescent light detecting optical

5   systems is fixed. This condition allows the pixels to be detected at the right positions from the above-described spacing, the above-described alignment mark detected positions by the CCD, and the detected position by the length-measuring devices, the pixels

10 corresponding to more minute division of the inside of each cell on the DNA chip. At this time, if the detection of 2 or more of the alignment mark positions indicates that the DNA chip has been rotated, this rotation is corrected using a rotation mechanism not

15 illustrated. Incidentally, the right position detection after this rotation correction is performed as necessary. Even if this rotation correction is not performed, if the amount of the rotation is small, it is also allowable to detect this rotation amount by the

20 above-described method and to correct the x, y coordinates in accordance with the rotation amount detected. Also, it is possible to correct and detect the above-described multi-spot lights by a microscopic rotation of the optical system.

25   As having been explained so far, the detection of the alignment marks and the length-measurement of the DNA chuck make it possible to detect the pixels in each cell on the DNA chip with the precise position-

ing accuracy. As a consequence, in accordance with the following method, it becomes possible to execute the fluorescent light detection toward all the pixels in the chip in sequence, at a high speed, and without

5 missing. A reference numeral 2A in FIG. 35 indicates an area detected by one scanning of the chuck. Here, the one scanning of the chuck is executed as follows: An operation of performing the irradiation with the M multi-spot array excitation lights, of performing the

10 sequential scanning by the amount of N pixels in the spot array direction, and of returning the spot array back to an original operational position is repeated, and at the same time, the chuck is scanned in the y direction. Namely, as illustrated in FIG. 36, first,

15 the pixels 2A101, 2A102, ··· ···, 2A10M on the DNA chip are illuminated and excited simultaneously. Next, the multi spots are displaced by the amount of one pixel-pitch ΔP by the above-described method, and this is continued in sequence.

20 When, in this way, the displacement by the amount of N(k) pixels is over, it turns out that the detection has been performed by the amount of MN(k) pixels in total, i.e., over a width of $NM\Delta P$, in a 1-dimensional array-like configuration. When the scan-

25 ning by the amount of N(k) pixels has been finished, the multi-spot array is returned back to the original position. During this, the DNA chip has been displaced by the amount of one pixel in the y direction, which

corresponds to a time-period from a time t_0 to t_1 of y_{st} in FIG. 39. Consequently, during the time-period from the time t_0 to t_1, the repetition of the above-described operation by the number of the y direction pixels

5 allows the fluorescent light detection to be finished toward all the pixels in the area 2A.

In the case where the above-described operation is performed by the DNA detecting apparatus of the embodiment in FIG. 1, the driving signal of the

10 multi-spot excitation lights by the AO deflector, or the displaced position of the spots is changed as is indicated by S_{px} in FIG. 38. When seen in more detail, the driving signal or the displaced position is step-displaced just like S_{B12} in FIG. 8. Also, the deflection

15 signal of the wavelength selection beam splitter is changed similarly to S_{px} in FIG. 38. The repetition of such changes from the time t_0 to t_1 permits all the area of the area 2A to be detected.

When the detection has been performed to the

20 end at the bottom of the area 2A (the time t_1), as is indicated by x_{st} in FIG. 40, the DNA chip chuck is step-displaced over the width of NM$\triangle$P in the x direction. After the step displacement, as is indicated by y_{st} after the time t_1 in FIG. 39, the chuck is scanned in an

25 opposite orientation in the y direction. Simultane-ously with this, just like S_{px} after the time t_1 in FIG. 38, the deflection signal of the AO deflector 12 and that of the wavelength selection deflecting beam

splitter 30 are scanned in an orientation opposite to the orientation at the time of detecting the area 2A (the time t_0 to the time t_1). This, as illustrated in FIG. 37, makes it possible to continuously detect an

5   area 2B subsequently to the detection of the area 2A. By repeating the above-explained operation alternately in the - orientation and the + orientation concerning the y direction of the DNA chip, it becomes possible to detect all the areas 2A to 2J on the DNA chip at a

10 high-speed.

Next, the explanation will be given below concerning a time needed to detect all the pixels on the DNA chip. Representing the cell number in the chip by L, each cell is divided into the N×N pixels so as to

15 avoid the influences of the foreign substance and the like. This makes the total detection pixel number in the chip equal to LN^2. Setting the simultaneous irradiation spot number of the multi-spot excitation lights and a time needed for the detection with the

20 multi spots to be M and Δt, respectively, and ignoring a time for the spot displacement and a time for the chuck movement in the x direction since these times can be assumed to be shorter as compared with the fluo-rescent light detecting time, the total fluorescent

25 light detecting time T is given by the following equation:

$$T = LN^2 \triangle t \: / \: M$$
$$= LN^2 \: / \: (M/\triangle t)$$

With reference to the number k of the scannings with the multi spots, a response time T_p of the wavelength separating beam splitter 30 needs to be $k\triangle t$ or larger. Also, the light-source power needs to be $\beta M/\triangle t$ or larger. Also, it is desirable that the spacing between the excitation multi-spot lights be equal to 5 or more in view of the signal-to-noise ratio.

At a longer fluorescent light wavelength of 670 nm, the quantum efficiency of the photomultiplier tube used for the fluorescent light detection becomes considerably small, i.e., 5 to 10%. Also, there exists a limit to the output of the laser light used for the excitation. Taking such conditions into consideration and in order to execute the detection as fast as possible, letting, for example, k (= N) = 10, M = 50, $\triangle t = 50 \mu s$, the cell number L = 1000×1000, and the in-cell division pixel number N = 5, T becomes equal to 25s. Namely, this result satisfies the condition of the fluorescent light detecting time that is smaller than $LN^2/(6\times10^5)$sec. Additionally, substituting the above-described values of L and N into this condition, the result is found to be 42 sec. This value satisfies the condition of executing the detection within 1 minute, including attachment/detachment of a sample chip for the use other than the fluorescent light

detection, inputting of the inspection conditions, and
outputting of the result.

Allowing the detection to be executed in 1
minute exhibits an outstanding effect in the case where
5   a lot of specimens to be detected are inspected. For
example, 1 minute becomes sufficient to execute an
inspection that has necessitated 5 minutes conven-
tionally. Accordingly, even if a preprocessing spends
somewhat a longer time, when the number of the
10 specimens becomes around 100, it becomes usually
possible to execute the preprocessing in parallel to
the inspections of the large number of specimens. This
makes it possible to shorten the detecting time by 5 or
6 hours.

15   The reasons why the present invention permits
such a high-speed and high-accuracy detection to be
implemented are as follows: The multi spots are used
as the excitation lights simultaneously. Moreover, in
the fluorescent light detection by each multi-spot
20 excitation light, the detection is executed in a state
of enlarging the spot spacing enough so that the
influences of the other excitation spot lights can be
suppressed up to the smallest possible degree.

FIGS. 41A, 41B, 41C, and 41D are diagrams for
25 illustrating an embodiment of the method and units for
obtaining the multi-spot lights according to the
present invention.

Using a method described later, substantially

parallel laser beams emitted from one light-source or a plurality of light-sources are formed into a plurality of (in the drawing, an even number) N/2 laser beams that are parallel to each other with a pitch of several

5 mm and that travel in the same direction, then being formed into N laser beams (N is an integer) by N/2 beam-doubling prisms 411, 412 and so on. As illustrated in an enlarged perspective view in FIG. 48B, the beam-doubling prism has a cross section of a triangle

10 and a cross section of a parallelogram. Here, the triangle has an oblique side forming an angle of substantially 45 degrees toward the light-emitting surface, and the parallelogram is in contact with this oblique plane and has a total reflection plane that is

15 parallel to this oblique plane.

A light launched into the one side of the parallelogram is reflected on a beam split plane of the triangle' oblique plane by the amount of substantially 50%, and the remaining substantially 50% passes through

20 the beam split plane. The light having passed through the beam split plane passes through the prism of the triangle without being subjected to any change. Meanwhile, the reflected light undergoes a total reflection on the other plane of the parallelogram, then passing

25 through the prism of the parallelogram. In this way, the light having passed through the triangle prism and the light having passed through the parallelogram prism are parallel to each other and have substantially the

same intensity. Also, the spacing between both of the

lights becomes equal to P. As a result, the beam

spacing between the adjacent multi beams has become

equal to P, which is one-half of the beam spacing 2P of

5   the laser beams 81e.

In this way, by a beam-doubling prism array

4e where such beam-doubling prisms are arranged by the

number of N/2 with the pitch of 2P, the laser beams 81e

are formed into the plurality of N laser beams 8e which

10 travel in substantially the same direction with the

pitch of P and the adjacent beams of which are

proximate to each other. The plurality of N laser

beams 8e, by being launched into a polarization

component 11e described later, are formed into a

15 plurality of 2N laser beams which travel in substan-

tially the same direction and the adjacent beams of

which are proximate to each other.

As illustrated in FIG. 42, the polarization

component 11e includes the 1st multi-spot lights-

20 doubling prism that has a polarization beam split plane

110e and transparent prisms 111e, 112e. Here, the

transparent prisms each have total reflection planes

1101, 1102 that are parallel to the polarization beam

split plane 110e and are positioned at distances of L1

25 and L2 from the polarization beam split plane 110e. On

a light-receiving plane of the polarization component

11e toward the N beams, there are located strip-shaped

convex lenses such as 21e, 22e, which result from

cutting a spherical flat convex lens into strip-shaped configurations, by the number of N in such a manner as to correspond to the positions of the respective N parallel beams. Here, the enlarged diagram and the

5 cross sectional enlarged diagram of the strip-shaped convex lenses are illustrated in FIGS 49A to 49D and FIG. 42, respectively. The laser beams having passed through an array 20e of these strip-shaped convex lenses, as illustrated in FIG. 42, become converged

10 lights each, then being launched into the polarization beam split plane in parallel to each other.

The respective converged beams are circularly polarized lights or linearly polarized lights polarized at 45 degrees toward the polarization beam split

15 incidence plane 110e. Consequently, as illustrated in FIG. 42, an S-polarized component of an incident light 82e is reflected on this plane, and a P-polarized component thereof passes through this plane. As illustrated in FIG. 42, a reflected converged light 822

20 passes through the prism 111e, then being launched into the plane 1101 parallel to the polarization beam split plane 110e at an incident angle of 45 degrees. A converged beam 822' that has undergone a total reflection on the plane 1101 is S-polarized, and thus is

25 reflected on the beam split plane 110e again. A reflected converged beam 822" passes through the prisms 111e, then going through.

Meanwhile, with respect to the P-polarized

component, a converged beam 821 having passed through the beam split plane 110e passes through the prism 112e, then being launched into the plane 1102 parallel to the beam split plane 110e at an incident angle of 45

5 degrees. The reflected light becomes a totally reflected converged beam 821', then being launched into the beam split plane 110e again. The incident light is P-polarized, and thus passes through the beam split plane by substantially 100%. This transmittance light

10 821" passes through the prism 111e, then going through.

The total reflection planes 1101, 1102 of the prisms 111e, 112e are positioned at the distances of L1 and L2 from the beam split plane. The values of L1 and L2 satisfy the following relation: $\sqrt{2}$ (L2 - L1) is

15 equal to P/2 that is one-half of the pitch P of the N parallel beams 8. Accordingly, when the lights having been separated by the beam split plane go through the polarization component, the two lights are shifted to each other by the amount of P/2. As a result, the N

20 converged beams with the pitch of P that have been launched into the polarization component 11e pass through the polarization component 11e in a state of being formed into 2N converged beams with the pitch of P/2.

25 On a light-emitting plane of the prism 111e in the polarization component 11e, as illustrated in FIG. 43, there is located a pinhole array mask 2 where pinhole apertures 211e having a diameter d are arranged

by the number of 2N with a pitch of P/2. The converged

lights pass through the pinhole apertures. Since the

portion other than the 2N pinhole apertures light-

shields lights, the pinhole array mask 2 light-shields

5 a stray light differing from the converged lights and

becoming a noise light. As a result, the lights having

passed through the pinhole array mask generate spot

array lights only from the pinhole portions.

The lights having passed through the pinhole

10 array 2e are linearly P-polarized lights and linearly

S-polarized lights alternately every adjacent spots.

Directly behind the pinhole array 2e, there is located

a quarter wave plate 3e. The quarter wave plate is set

in such a manner that the optical axis forms 45 degrees

15 toward the P-polarized and S-polarized directions.

Consequently, the P-polarized and the S-polarized

lights having passed through the quarter wave plate 3e

become right-handed and left-handed circularly

polarized lights, respectively.

20 The 2N parallel circularly polarized lights

having passed through the quarter wave plate 3e are

launched into a polarization component 12e. The

polarization component 12e is a polarization beam

splitter the structure of which is substantially the

25 same as that of the above-described polarization

component 11e. Namely, a beam split plane 120e permits

a P-polarized light to pass through and causes an S-

polarized light to be reflected. Also, the values of

L1' and L2' satisfy the following relation: $\sqrt{2}$ (L2' - L1') = $\pm$P/4. Here, L1' denotes a distance between the beam split plane and a total reflection plane 1201 of a prism 121e through which the S-polarized light passes

5 after having been reflected by the beam split plane. L2' denotes a distance between the beam split plane and a total reflection plane 1202 of a prism 122e through which the P-polarized light passes after having been reflected by the beam split plane. By providing the

10 above-explained configuration, the 2N parallel beams with the pitch of P/2 that have been launched into the prism 12e are formed into mutually parallel 4N diverged beams with a pitch of P/4, then being emitted from the prism 12e.

15   As described above, the lights emitted from the 2N spots existing on the pinhole array mask are formed into the above-described 4N multi-spot lights by the polarization component 12e. At this time, the polarization component 12e makes the 4N multi-spot

20 lights look as if the multi-spot lights were emitted from the surface of the mask 2e. Namely, when seen from the side on which the multi-spot lights have already passed through the polarization component 12e, the 4N multi-spot lights are formed on the surface of

25 the mask 2e, then being emitted therefrom in the state of having become the diverged lights. This phenomenon is caused by a mirror image effect by the reflection planes 120e, 1201, and 1202.

As one example, assuming that the number of N is equal to 16 and the pitch P is equal to 1.6 mm, in the above-described embodiment, the number of the multi-spot lights and the pitch become equal to 64 and

5 0.4 mm, respectively. By launching the multi-spot lights into an objective lens of an infinite focal point microscope through an image-forming lens, assuming that the magnification of the optical system is 20 times, it becomes possible to irradiate a sample, i.e.,

10 a target to be inspected, with 2 μm-diameter spots with a 20 μm pitch by the number of 64 simultaneously. This will be explained in detail later.

In the above-described embodiment in FIG. 41A, the N/2 multi beams are launched into the 2nd

15 beam-doubling prism array 4e. When N is equal to 8, if the laser light-source is not the He-Ne laser type producing a large output but a large intensity is required as the irradiation light, it is sufficient to prepare, for example, 8 He-Ne lasers and to launch the

20 lasers into the 2nd beam-doubling prism array 4e with a 1. 6 mm pitch. Also, it is sufficient that 8 semicon-ductor lasers are used and are formed into mutually parallel beams, thereby configuring 8 beams as is the case with the above-described He-Ne lasers so as to

25 launch the lasers into the 2nd beam-doubling prism array 4e. In this case, since the pitch P is set to be 1.6 mm, the spacing 2P between adjacent beams of the 8 laser beams becomes equal to 3 mm, which is greater

than the beam diameter of the He-Ne laser light, i.e.,
1 mm. Accordingly, it is possible to launch the lasers
in parallel to each other from the 8 laser light-
sources.

5   In this way, even in the state where, by the
2nd beam-doubling prism array, the pitch is made equal
to the spacing P that is less than 4 times as great as
a diameter d of the beams emitted from the laser light-
source, i.e., 4d or less, causing the beams to be
10 launched into the multi-spot light forming unit using
the polarization component allows the spacing to be
made equal to 1/2 thereof. Also, using the polariza-
tion component a plurality of n times allows the
spacing to be made equal to $1/2^n$ thereof. In this way,
15 it becomes possible to execute the conventionally
difficult following task: Converting the plurality of
N laser beams, which are launched with the pitch that
is less than 4 times as great as the beam diameter d,
into the $2^{M'}N$ multi laser beams with the pitch of $P/2^{M'}$,
20 thereby allowing the multi-spot lights to be formed
with a high-efficiency.

          FIG. 44 is a diagram for illustrating another
embodiment for forming the multi-spot lights according
to the present invention. In the present embodiment,
25 the N multi beams to be launched into the polarization
component are formed using one laser light-source. For
example, in the case where, like YAGSHG laser, the
output of the light-source is large, the one laser

light-source is effective enough. In such a case, as illustrated in FIG. 44, in addition to the 2nd beam-doubling prism denoted by 4e in FIG. 41A, the 2nd beam-doubling prisms the dimensions of which are 2 times, 4

5   times, and 8 times the dimension of the above-described 2nd beam-doubling prism 4e are arranged in a cascaded manner at n = 4 stages in total.

Namely, one beam 80e emitted from one light-source is launched into the 8-times-dimensioned beam-

10 doubling prism 441, thereby obtaining 2 beams 801 and 802 with a spacing of 8P. The 2 beams are launched into the 4-times-dimensioned beam-doubling prisms 431 and 432, thereby obtaining 4 beams 8011, 8012, 8021, and 8022 with a pitch of 4P. The 4 beams are launched

15 into the 2-times-dimensioned beam-doubling prisms 421, 422, 423, and 424, thereby obtaining 8 beams with a pitch of 2P. The 8 beams are launched into the previously-mentioned 8 beam-doubling prisms 411, 412, 413, 414, 415, 416, 417, and 418 illustrated in FIG.

20 41B, thereby obtaining 16 beams with a pitch of P. This allows the 16 beams with the 1.6 mm pitch to be obtained from the one beam emitted from the one laser light-source.

The method explained in the above-described

25 embodiment makes it possible to form the multi-spot lights having a total energy that is 70% or more of the energy of the laser beam emitted from the laser light-source, which was impossible in the prior art.

Moreover, taking the following measures makes it possible to form the multi-spot lights having a total energy that is 90% or more of the energy of the laser beam emitted from the laser light-source: Equip-

5 ping the optical components with an antireflection coat, providing the optical components with a multi-layer coat for lowering the loss at the time of the beam separation, performing positioning of the incident lights toward the pinhole array, or the like.

10   Furthermore, the method explained in the above-described embodiment makes it possible to restrain the variation in the respective spot energies among the multi-spot lights within ±20%, which was impossible in the prior art.

15   Still further, taking the following measures outstandingly lowers a variation in the beam intensity caused by the reflection and the interference of the beams on the optical component surfaces, thereby making it possible to form the multi-spot lights that allow

20 the variation in the respective spot energies among the multi-spot lights to be restrained within ±10%: Submitting conditions on the multi-layer coat at the time of the beam separation and controlling the manufacturing thereof, or bonding, with an optical

25 adhesive, the mutually adjacent components out of the optical components such as the polarizer 11e in FIG. 41A, the strip-shaped convex lens array 20e the detail of which is illustrated in FIG. 41C, the pinhole array

2e, the wavelength plate 3e, and the polarizer 12e.

Incidentally, although, in the above-described embodiments, the explanation has been given concerning the case of forming the 64 or 16 multi-spot

5   lights, the present invention is not limited thereto and is effective in the case of $N = 2$, i.e., the case of obtaining 8 or more of the multi-spot lights.

Next, referring to FIG. 45, the explanation will be given below concerning an embodiment where the

10 beams obtained by the multi-spot light forming methods explained so far are applied to the fluorescent light detection, especially to the DNA inspection using the fluorescent light detection.

In FIG. 45, numerals 8001, 8002, 8003 ⋯ ⋯

15 denote laser light-sources, and include 8 lasers in total that are not illustrated though. The beams emitted from the respective laser light-sources are directed into one direction in parallel to each other with a pitch of about 1.6 mm by mirrors 8011, 8012,

20 8013 ⋯ ⋯ that are several mm in size. The 8 beams are obtained by the above-described method from the polarization component 12e as if the 64 multi-spot lights were emitted from the mask surface 3e with the 0.4 mm pitch. The 64 multi-spot lights pass through an

25 image-forming lens 61e, a mirror 63e, a wavelength selection beam splitter 70e, then being illuminated onto an inspection plane 51e of a DNA chip 5e through an objective lens 60e.

On the surface of the DNA chip, there has been performed in advance the probing of a DNA determined for each of predetermined positions. Meanwhile, a target DNA obtained by adding a fluorescent material

5 to a DNA formed by refining and amplifying a living body is poured onto the above-described probe DNA. Then, if the nucleotide sequence of the probe DNA and that of the target DNA correspond to each other, they are hybridized with each other. As a result, the

10 fluorescent material-added target DNA is coupled with the probe DNA.

There exist various types of fluorescent materials to be added to the target DNA. The fluorescent material in the present embodiment absorbs the

15 He-Ne laser light at the wavelength of 633 nm and emits the fluorescent light at the wavelength of 670 nm. The fluorescent light is received by the objective lens 60e, and the transmittance light is reflected by the wavelength selection beam splitter 70e, thereby being

20 guided into a detecting optical system. The wavelength selection beam splitter 70e is a multi-layer coat beam splitter for permitting the 633 nm light to pass through and causing the 670 nm light to be reflected.

The fluorescent light reflected at the beam

25 splitter 70e is reflected at a mirror 71e, then passing through an image-forming lens 62e. The image-forming lens 62e is configured as a telecentric optical system, so that primary light-rays of the fluorescent lights,

which have been generated from the respective multi-spot lights with which the fluorescent materials on the DNA chip surface are irradiated, travel in parallel to each other after passing through the image-forming lens

5   62e. As a consequence, at an incident angle that is common to all the fluorescent lights, a fluorescent light generated from whatever multi-spot light is launched into a wavelength separating beam splitter reflecting the fluorescent lights at the above-

10   described wavelength. This condition permits the 633 nm excitation light to pass through the beam splitter in a state of being scarcely reflected. Here, the 633 nm excitation light is a noise component contained slightly in amount in the fluorescent light generated

15   from whatever multi-spot light. Namely, although the 633 nm excitation lights reach a wavelength separating beam splitter 72e slightly in amount, the excitation lights are caused to pass through the beam splitter. As a result, only the fluorescent lights are reflected,

20   then traveling toward detectors 91, 92.

       The above-described excitation light, which has been reflected on the DNA chip and then travels toward the detector side, has a light intensity that is stronger by several orders or more in comparison with a

25   light intensity of the fluorescent light generated. On account of this, as described above, it is impossible to separate the excitation lights using the wavelength separating beam splitter 70e alone. In particular, the

incident angles of the excitation lights into the beam splitter 70e differ, depending on the positions of the excitation light spots. Accordingly, it is impossible for any spot to eliminate the corresponding excitation

5 light equally. In contrast to this, as explained above, the wavelength separating beam splitters 72e, 73e located between the telecentric image-forming lens 62e and the detectors 91, 92 are capable of reflecting the light from whatever spot position on the same

10 incidence condition. The elimination of the excitation lights like this and interference filters 74e, 75e light-shield the excitation lights almost completely, allowing only the fluorescent lights to be taken into the detectors.

15   The detectors 91, 92, which are multi-channel photomultiplier tubes, have 32 light-receiving apertures each. Spot images of the fluorescent lights are separated into two by a right-angled mirror 76e located in front of the photomultiplier tubes 91, 92,

20 then being image-formed at substantially the positions of the light-receiving apertures of the photomultiplier tubes. There exist pinhole arrays in front of the light-receiving apertures of the photomultiplier tubes. The pinhole arrays have apertures and a pitch that are

25 substantially equal to the dimensions of spot images that are image-formed by the DNA chip-irradiating 2 μm-diameter and 20 μm-pitch excitation multi-spot lights with a magnification M of the objective lens and the

image-forming lens. Namely, the pinhole arrays have the apertures 2M μm in diameter and the pitch of 20M μm. The pitch of 20Mμm is equal to a light-receiving aperture pitch of the photomultiplier tubes.

5   This corresponds to performing the simultaneous irradiation with the multi-spot light array and performing the confocal point detection toward the generated fluorescent lights, thereby making it possible to execute the fluorescent light detection
10 having a high signal-to-noise ratio. The 64 signals obtained are amplified by a circuit not illustrated, and are converted into digital information by an A/D (analogue/digital) converter, thus being transferred to a CPU (processing circuit) not illustrated. Then, data
15 on the fluorescent light intensities at the irradiation positions is stored.

        Stages 501, 502 are driven, thereby determining the fluorescent light intensities at the different positions in sequence in accordance with the above-
20 described method. In order to execute the fluorescent light detection having a high signal-to-noise ratio, the pitch of the irradiation spots is made equal to several to tens of times the diameter of the irradiation spots. Accordingly, so as to accomplish the
25 entire surface detection all over the target to be inspected, the detection is performed by driving the xy stage not only in a direction perpendicular to the arrangement of the multi-spot lights but also in the

arrangement direction.

FIG. 46 is a diagram for illustrating an embodiment in the present invention. The parallel beams 8e emitted from the one laser light-source or the

5 plurality of laser light-sources are the circularly polarized lights or the linearly polarized lights polarized at 45 degrees toward the paper surface. One beam among the plurality of parallel beams 8e is launched into the one lens 21e within the array 20e of

10 the strip-shaped convex lenses and becomes the converged beam, then being launched into a birefringent material such as calcite. A polarized component of an ordinary ray thereof travels straight without being altered, and a polarized component of an extraordinary

15 ray thereof is refracted. A thickness of this polarization component is set so that a spacing between the ordinary ray and the extraordinary ray at the time of having passed through the polarization component becomes equal to one-half of the arrangement pitch P of

20 the plurality of N incident beam lights 8e. As a result, after having passed through the polarizer 11e', 2N converged beams travel with a pitch of P/2 in parallel to each other.

In the respective beams, the polarized

25 directions in their linearly polarized states differ from each other alternately by 90 degrees. The respective beams are caused to pass through the quarter wave plate 3e having the optical axis that forms 45

degrees toward the polarized directions, or a half wave plate 3e having an optical axis that forms 22.5 degrees toward the polarized directions, thereby obtaining circularly polarized lights or linearly polarized

5 lights having polarized directions polarized at ±45 degrees toward the paper surface. These 2N beams are launched into a polarizer 12e'. The polarizer 12e' is a calcite the thickness of which is one-half of that of 11e'. The beams have passed through the polarizer

10 12e', which obtains 4N converged beams with a pitch of P/4.

These lights are caused to pass through a wavelength plate 3e' that is the same as the above-explained wavelength plate, thereby becoming circularly

15 polarized lights or linearly polarized lights polarized at 45 degrees toward the paper surface. These multi beams are launched into a plate 2e including a multi-aperture array that has a pitch and an aperture diameter. Here, the pitch is equal to the pitch of the

20 multi beams, and the aperture diameter is substantially equal to a converged diameter determined by the lens 21e and the diameter of the beams launched into the lens. This makes it possible to obtain noise-free multi-spot lights including only the lights having

25 passed through the apertures.

FIG. 47 is an embodiment-illustrating diagram for indicating a multi-spot light forming method used for the confocal point detection in the present

invention.   In FIG. 47, the same reference numeral as
that in FIGS. 41A to 41D denotes the same component.
The beams emitted from 1e or the plurality of laser
light-sources are formed into the 8 parallel beams,
5 then being formed into the 16 parallel beams by the 2nd
beam-doubling prism 4e.   The 16 parallel beams are
formed into the converged beams by the convex lens
array 20e.

Then, the 1st multi-spot lights-doubling
10 prisms 11e, 12e form the 64 multi-spot lights.   At this
time, the 64 multi-spot lights are obtained as if the
64 multi-spot lights were emitted effectively from the
apertures of the mask 3e by the operation of the 1st
multi-spot lights-doubling prism 12e.   A quarter wave
15 plate 3e′ is attached to a light-emitting surface of
the multi-spot lights-doubling prism 12e.   Thus, the 64
multi-spot lights become circularly polarized lights,
then being launched into a polarization component 11e′
composed of the calcite.   The polarizer 11e′, a quarter
20 wave plate 3e″, a polarizer 12e″, a quarter wave plate
3e″′, a polarizer 13e′, and a quarter wave plate 3e″″
are based on the same beam 2-splitting method as that
explained in FIG. 46.   Thus, in the polarizers 11e′,
12e′, and 13e′, the thickness of each calcite becomes
25 thinner just as t, 1/2t, and 1/4t.   Also, the direction
in which the beams are going to split is the y direc-
tion.   Accordingly, the 64 spot lights are generated in
the x direction by the 1st multi-spot lights-doubling

prisms 11e, 12e, and 8 spot lights are generated in the
y direction by the beam 2-splitting method using the
calcite. Consequently, as illustrated in FIG. 48, 64×8
spot lights are generated in total.

5   FIG. 49 indicates a confocal point detecting
method using the above-described 64×8 multi-spot
lights. In FIG. 49, the same reference numeral as that
in the other drawings denotes the same component. The
8 beams emitted from a laser light-source and formed by
10   an 8-beam optical system 800 are launched into the 64×8
multi-spot light-forming optical system 100e illus-
trated in FIG. 47. Then, in accordance with the above-
described method, the 64×8 multi-spot lights illus-
trated in FIG. 48 are being emitted effectively from
15   the mask surface 3e.

       These multi-spot lights are reduced in size
and are projected onto a surface of a target 5e' to be
measured, using a half mirror 70e', the image-forming
lens 61e, and the objective lens 60e. The projected
20   multi-spot lights are reflected on the surface to be
measured, and pass through the objective lens 60e, the
image-forming lens 61e, and the half mirror 70e', then
being image-formed onto a surface of a 64×8-pinhole
mask 901' located directly in front of an image-pickup
25   surface of a detector 90'. The lights having passed
through the pinholes reach the image-pickup surface of
the detector. If the surface of the to-be-measured
target coincides with an image-forming plane of the

projected multi-spot lights, the reflected light are image-formed on the pinholes of the pinhole mask, thereby making it possible to obtain the strong detected values. If the surface of the to-be-measured

5   target does not coincide with the image-forming plane, out-of-focused spread lights reach the pinholes of the pinhole mask 901', and accordingly the intensities of the lights having passed through the pinholes become lower. Consequently, by executing the above-described

10   detection with the to-be-measured target being displaced in an up-and-down direction by z-stage 503' shown in Fig. 49, it becomes possible to obtain the heights at the positions of the respective spots from their z direction values from which the detection

15   maximum intensities at the respective positions can be obtained.

In the above-explained confocal point detecting method in FIG. 49, when the target to be measured is fixed in the x, y directions, if, for example, the

20   spot diameter is 1 μm and the spot spacing is 8 μm on the surface to be measured, only information about the 64×8 points with a 8 μm spacing can be obtained. Accordingly, it is required to detect the confocal point images at the other positions in sequence by

25   displacing xy stage 501', 502'. FIG. 50 is an embodiment-illustrating diagram in the present invention, where there is indicated a method of obtaining the 2-dimensional confocal point images. The driving

directions x', y' of the xy stage in FIG. 49 and the

arrangement directions of the multi-spot lights to be

projected onto the to-be-measured target are shifted

slightly to each other as illustrated in FIG. 50.   In

5   the case of the 64×8 multi-spot lights, an angle θ

formed between these two directions is set so that $\tan\theta$

becomes equal to 1/8.   On account of this shifting,

scanning the stage in the y' direction results in

irradiating empty positions between a spot 011 and a

10   spot 021 with 7 spots, i.e., 012, 013, ∙∙∙ ∙∙∙ , 018.

This means that the entire surface scanning is

performed all over the 1 μm spots with a 1 μm spacing

placed therebetween.

The use of the above-described method

15   irradiates the to-be-measured target with the multi-

spot lights having high light intensities, and

accordingly it becomes possible to detect the 64×8 spot

positions in a short time.   Thus, one scanning toward

the samples is performed in the y' direction at a high-

20   speed by the y stage and after the scanning, the stage

is displaced slightly in the z direction, and the

scanning is performed again.   This operation is

repeated, thereby detecting confocal point signals at

the respective heights in sequence at a high-speed.   In

25   this way, a height at which the intensity is a maximum

at each point is determined from the 2-dimensional

intensity data at each point obtained at each height z.

This makes it possible to execute the height measure-

ment by the high-speed confocal point detecting method.
Incidentally, setting the width of the above-described
scanning to be equal to k times the pitch p of the
multi-spot lights results in obtaining the 2-

5   dimensional height information where the pixel number
in the x direction is equal to $64 \times 8 = 512$ and the pixel
number in the y direction is equal to 8k.

Next, the explanation will be given below
concerning another embodiment for forming the multi

10 spots.

Here, taking the DNA probe array as an
example, the explanation will be given concerning an
apparatus and a method for measuring an intensity
distribution of the fluorescent lights. The fluo-

15 rescent lights are emitted from the fluorescent markers
for marking the target DNAs trapped in the state of
being hybridized with the DNA probes fixed onto the
large number of microscopic reaction areas on the DNA
probe array. Additionally, it is needless to say that

20 the present invention is not limited to the inspection
of the DNA, but is similarly applicable to the inspec-
tion and analysis of the other living body samples such
as a fluorescent material-marked RNA, oligonucleotide
and protein.

25 The DNA probe array to be detected is formed
in the following way, for example: First, a washed
glass substrate (such as a slide glass) is treated
using a silane coupling agent (aminopropyltriethoxy-

silane), thereby introducing an amino group onto the surface. Next, an aldehede group-introduced oligo-nucleotide is dropped on microscopic areas so as to be reacted, thereby fixing the oligonucleotide on the

5   glass substrate. A fluorescent marker-attached DNA is prepared independently as a specimen, i.e., a target to be inspected. Then, the fluorescent marker-attached DNA is spotted onto the oligonucleotide-fixed areas on the substrate so as to be trapped on the substrate by

10   being hybridized therewith, thereby preparing the array to be measured. Incidentally, in order to fix the oligonucleotide on the glass substrate, the following methods are allowable: The surface treatment of the slide glass is performed using poly positive ions such

15   as polylysine, thereby causing the electrostatic bond with electric charges of the DNA. Besides, the oligonucleotide is synthesized directly on the glass substrate, using a photolithography technique and a solid phase synthesizing technique. Being not limited

20   to the above-described forming method, the DNA probe array can be formed by various well-known methods, and is measurable in much the same way.

         FIG. 51 is a schematic diagram for illustrat-ing the entire configuration of one embodiment of a

25   fluorescent marker distribution measuring apparatus (DNA probe array measuring apparatus) according to the present invention. The apparatus includes a main body 1f of the DNA probe array measuring apparatus, a data

processing/controlling unit 19f, and a monitor 21f. A

substrate (DNA probe array) 2f is fixed on a XYZ

driving unit 20f. A light from a light-source 10f of

laser or the like is caused to pass through a multi

5   light-source distribution unit 11f, thereby being

emitted as a plurality of lights (100a, 100b, ···) that

are located on straight lines equally and uniformly.

These lights are collimated by a lens 12f, and are

reflected downward by a dichroic mirror 13f, and are

10 converged by an objective lens 14f, finally forming

excitation spots (101a, 101b, ···) onto the substrate 2f.

In the drawing, although only 2 of the lights and 2 of

the excitation spots are illustrated for concern with

the paper space, 64 excitation spots are formed

15 actually. The larger the number of the excitation

spots is made, just like 16, 32, 64, ···, the more

effective it becomes to implement the high-speed and

high-sensitivity characteristics. Namely, if the total

detecting time is the same, the larger the number of

20 the spots becomes, the more likely it is to be able to

lengthen the irradiating time per excitation spot and

to be able to enhance the sensitivity. Also, if the

irradiating time per excitation spot is the same, the

larger the number of the spots becomes, the more likely

25 it is to be able to shorten the total detecting time.

In this case, 64 times speeding-up becomes executable.

Emitted lights (fluorescent lights, scattered

light, reflected lights) generated from the excitation

spots on the substrate 2f are converged again by the
objective lens 14f, and are caused to pass through the
dichroic mirror 13f (a 2-color property mirror for
causing wavelength components of the excitation lights

5   to be reflected and permitting longer wavelength
components of the fluorescent lights to pass through),
thereby extracting the fluorescent light components.
Moreover, the fluorescent light wavelength components
pass through an interference filter 15f deigned to

10   permit the fluorescent light wavelength components to
pass through. In addition, the fluorescent light
components are converged by a lens 16f, thereby causing
images of the excitation spots (101a, 101b, ···) to be
image-formed as fluorescent light images (102a, 102b,

15   ···).

A multi-detection unit 17f substantially
simultaneously detects fluorescent light intensities
generated from the plurality of excitation spots.
Light signals from the multi-detection unit 17f are

20   converted into numerical values by an A/D conversion
unit 18f in harmony with a timing from the data
processing/controlling unit 19f, then undergoing the
processing such as various arithmetic operations by the
data processing/controlling unit 19f. Also, the data

25   processing/controlling unit 19f controls displacement
of the XYZ driving unit 20f, and constructs a 2-
dimensional distribution of the fluorescent light
intensities on the substrate 2f in combination with the

XY driving information, then displaying the 2-dimensional distribution on the monitor 21f. This makes it possible to detect the fluorescent light intensity distribution on a predetermined area surface

5   of the substrate 2f, the total fluorescent light intensities of the plurality of microscopic areas provided on the substrate 2f, and so on. Accordingly, it becomes possible to calculate a quantity of the specimen DNAs coupled with the fixed oligonucleotide.

10   Here, on the surface of the substrate 2f, the excitation spots are set to be 2 μm in diameter and the spacing between the excitation spots is set to be about 20 μm. It is desirable to make an adjustment so that the size of the excitation spots becomes equal to 1/an

15 integer-th of the spacing between the excitation spots. In the case of forming the 64 excitation spots, the spacing between both ends of all the spots becomes equal to 1.26 mm. Taking this into consideration, as the objective lens, the one satisfying the following

20 conditions has been manufactured and used: The field-of-view 1.3 mm, the numerical aperture NA = 0. 70 (in order to enhance a fluorescent convergence efficiency), and (taking into consideration the case where the irradiation is performed from the back surface of the

25 glass) a distance between the lens tip and the sample position 1.3 mm.

In order to measure the fluorescent lights all over the entire surface of an attention-paid area

of the substrate 2f, the XYZ driving unit 20f is
displaced in the following way: FIG. 65 is an
explanatory diagram for explaining an example of the
displacement of the XYZ driving unit 20f. The

5 arrangement direction of the excitation spots is taken
as an x axis. As illustrated in the drawing, the
excitation spots S0, S1, S2, ···, S63 are located in
their initial positions in such a manner that the
position of the excitation spot S0 becomes (0, 0),

10 i.e., (x, y) = (0, 0). An area 700 is the attention-
paid area to be measured which includes the oligo-
nucleotide-fixed areas on the DNA probe array. In
order to scan the entire surface of the area 700, the
stage is displaced in a 2-dimensional manner. In the

15 drawing, an arrow illustrates an orbit along which the
excitation spot S0 is moved relatively to the area 700.

First, all the width of the area 700 is
scanned in the y direction. Then, the y positions are
returned back to 0, and the spots are shifted by the

20 amount of the excitation spot diameter (in the present
example, 2 μm) in the x direction. All the width of
the area 700 is scanned again. This operation is
repeated a plurality of times by the time the excita-
tion spot S0 reaches the first position of S1. In the

25 drawing, for simplicity, the scale-reduced illustration
is given in such a scale that the spacing between the
excitation spots is equivalent to 8 μm, and there is
illustrated a manner where the positions in the x

direction are changed 4 times so as to scan all the width in the y direction. Incidentally, in the case where the excitation spots are equal to 2 μm in diameter and the spacing between the excitation spots

5 is equal to 20 μm, it turns out that the above-described operation has been repeated 10 times. This makes it possible to detect the entire surface of the area the width of which is x = 0 to 1280 μm. The next operation is as follows: The y positions are returned

10 back to 0 and the spots are shifted by 1260 μm in the x direction, thereby displacing the spots to an area that is adjacent to the area where the scanning has been finished using the 64 excitation spots. Namely, the spot S0 is displaced to the position corresponding to

15 S64, and the above-described operation is repeated. This is repeated, thereby scanning the entire surface of the area 700.

FIGS. 66A, 66B are diagrams for illustrating displacement curves of x, y axes of the XYZ driving

20 unit 20f. FIG. 66A illustrates a displacement state of the XYZ driving unit in the x direction, and FIG. 66B illustrates a displacement state of the XYZ driving unit in the y direction. As is illustrated in the drawing, the operation of the present driving unit is

25 characterized by repeating at least 2 types of displacement patterns in the x direction, i.e., the arrangement direction of the excitation spots, thereby scanning the entire surface of the to-be-measured area.

Here, the 2 types of displacement patterns are step-like displacements with a microscopic width of 2 μm at n times (in the present example, 10 times) and a subsequent step-like displacement with a longer width

5   at one time. This characteristic results from the existence of a limit to the objective lens' field-of-view size and the existence of a restriction to the spacing between the plurality of excitation spots. If the spacing between the excitation spots is made equal

10 to a width obtained by substantially equally dividing the entire surface of the to-be-measured area by 64, it becomes unnecessary to employ the displacement method as described above. Namely, in the x direction, it is all right to repeat the step-like displacements with

15 the microscopic width of the excitation spot diameter. In this case, however, the spacing between the excitation spots at both ends gets large, becoming substantially equal to a width of the entire surface of the to-be-measured area. This width exceeds the field-of-

20 view width of a common objective lens (i.e., an objective lens being able to form the excitation spots of about 2 μm), thereby making it difficult to execute the measurement. If there should exist an objective lens having a sufficiently large field-of-view, the

25 numerical aperture will become smaller or such an objective lens will be an extremely expensive one, either of which is unrealistic. Namely, the scanning method illustrated in FIGS. 65, 66A, and 66B is a

method suitable for scanning the entire surface of the to-be-measured area with the use of the plurality of excitation spots.

5   Also, as timings of the data collection at the time of the scanning, there exist 2 timings, i.e., the timing at the time of the displacement in the x direction and the timing at the time of the displacement in the y direction. In principle, either of the timings makes it possible to obtain the information on

10   the entire surface. However, it is advantageous to collect the data in harmony with the displacement in the y direction in particular. This is due to the following reasons: A stroke of the displacement at one time is longer in the y direction, and accordingly the

15   connection with the adjacent pixel is more clear, and thus it is easy and secure to reconstruct an image from the measured data.

       Next, the explanation will be given below concerning the configuration of the multi light-source

20   distribution unit 11f. When the size of the excitation spots is 2 μm in diameter and the spacing between the excitation spots is 20 μm, at a light-emitting end of the multi light-source distribution unit 11f, the following is required: Assuming that a coupled

25   magnification including the objective lens is 20 times, the lights need to be emitted from 64 portions under the condition that, at the light-emitting end, the size of the light spots is 40 μm in diameter and the spacing

between the excitation spots is 400 μm. Since it is usually impossible to arrange the light-sources with the spacing of 400 μm, the following configurations have been constructed in the present invention. These

5   configurations will be explained below, using FIGS. 52 to 57, and FIGS. 67 to 69.

FIG. 52 is a diagram for illustrating the configuration of an embodiment of a multi light-source distribution unit 11f for splitting lights emitted from

10   n laser light-sources (2 of them are illustrated in the drawing) into 4n lights. Lights emitted from laser light-sources (10a, 10b) are each converged by lenses (200a, 200b), thereby being introduced into optical fibers (201a, 201b). Each optical fiber has 2 branch-

15   ing portions halfway, by which each of the converged lights is split into 4 lights. Accordingly, the excitation lights can be split just like 100a1, 100a2, ··· 100b4. Incidentally, as the branching portion, it is possible to select not only the 2-branching type as

20   illustrated in the drawing but also the other various types such as a 4-branching type. Also, in such a branching, there is employed a fiber that allows a light to be split equally. For example, light-emitting ends of optical fibers the core diameter of which is

25   equal to about 40 μm are arranged with the spacing of 400 μm on a straight line, then projecting the lights in a 1/20th scale-reduced state. This makes it possible to implement the configuration in the present

embodiment.

FIG. 53 is a diagram for illustrating the configuration of another embodiment of the multi light-source distribution unit 11f for simply arranging

5   lights emitted from m laser light-sources (4 of them are illustrated in the drawing) with the spacing of 400 μm on a straight line. The case m = 4 is illustrated in the drawing. Lights emitted from the laser light-sources (10a, 10b, 10c, 10d) are each converged by

10 lenses (200a, 200b, 200c, 200d), thereby being intro-duced into optical fibers (202a, 202b, 202c, 202d). Light-emitting ends of the optical fibers are arranged with the spacing of 400 μm on a straight line. This makes it possible to implement the configuration in the

15 present embodiment concerning the lights (100a, 100b, 100c, 100d) regardless of the size of the laser light-sources with the spacing of 400 μm.

FIG. 54 is a diagram for illustrating the configuration of another embodiment of the multi light-

20 source distribution unit 11f utilizing the birefringent material (for example, calcite, and the other materials are also usable). As illustrated in the drawing, birefringent materials 300f, 302f such as calcite and quarter wave plates 301f, 303f are combined with each

25 other in a sequence of, from the laser light-source side, the birefringent material 300f, the quarter wave plate 301f, the quarter wave plate 302f, and the quarter wave plate 303f, thereby forming a splitting

component 310f. A laser light emitted from a laser light-source 10a is launched into the above-described component through a lens 200a. At this time, the polarization surface of the laser light has been

5 inclined by 45 degrees, or the laser light has been converted into a circularly polarized light by being caused to pass through a quarter wave plate on the way. The laser light launched into the birefringent material 300f is double-refracted, thereby being separated into

10 an ordinary ray and an extraordinary ray. A length of the birefringent material 300f is adjusted so that the two rays are away from each other by a distance w1. These lights are converted into circularly polarized lights by being caused to pass through the quarter wave

15 plate 301f further, then being launched into the birefringent material 302f. Each of the 2 lights into which the light has been split is further split into 2 lights similarly. A length of the birefringent material 302f is adjusted so that the distance between

20 the 2 lights becomes equal to w2. Making the adjust-ment so that w1 becomes 2 times as long as w2 allows the laser light to be split with an equal spacing. Incidentally, in order to let w2 = 400 μm, it is all right to set the lengths (in the optical axis direc-

25 tion) of the birefringent materials 300f and 302f to be about 8 mm and about 4 mm, respectively (in the case where the birefringent materials are calcite and the crystalline axes form 45 degrees toward the optical

axes). Additionally, the lengths of the respective

birefringent materials are varied, depending on the

kind of the materials, the wavelength, and inclinations

of the crystalline axes. When the directions of the

5   crystalline axes are identical to each other, as

described earlier, the length of the birefringent

material is set to be 1/2th of that of the birefringent

material at the preceding stage.

    This configuration allows the 1 laser light

10   to be split into the 4 laser lights equally and with

substantially the same intensity, and permits the

light-emitting ends to be located on a straight line.

Thus, locating 16 laser lights with a spacing of 0.4×4

= 1.6 mm allows 64 light spots to be located with a

15   spacing of 0.4 mm on a straight line.. Incidentally,

setting a focal point position of the lens 200a to be

the position of pinholes 304f makes it possible to

eliminate extra lights such as scattered lights.

Accordingly, it becomes possible to make beautiful the

20   configuration of the excitation spots on the substrate

2f, thereby permitting the resolution to be enhanced.

Furthermore, the focal point position can be utilized

as one focal point position for measuring the confocal

point.

25   Also, although the splitting component 310f

in the drawing has the configuration of executing the

splitting with the 2 stages, it is also possible to

employ 3 stages, 4 stages, and 6 stages. In these

cases, it turns out that one laser light is split into 8, 16, and 64 lights, respectively, and the number of the laser lights required to obtain the 64 light spots can be made smaller like 8, 4, and 1. For example, in

5 the case of a 6-stage configuration where there are employed the birefringent materials the respective lengths of which are 32, 16, 8, 4, 2, and 1 mm in sequence from the light-incoming side, it is possible to split the 1 laser light into 64 lights with a

10 spacing of 0.1 mm.

FIG. 55 is a diagram for illustrating the configuration of another embodiment of the multi light-source distribution unit 11f. The components mainly includes 4 trapezoid-shaped glass prisms 361, 362, 363,

15 and 364 and a quarter wave plate 367. The glass prisms 361, 362, 363, and 364 are connected to each other as illustrated in the drawing, and connected planes 365, 366 are polarization beam splitters. The respective glass prisms differ slightly in their dimensions.

20 Thus, an incident light in a circularly polarized state is split into 2 lights on the connected plane 365, then becoming proximate to each other after the total reflection. At this time, making the optical path lengths differ from each other allows 2 parallel laser

25 light-fluxes to be obtained. When causing the 2 lights to be launched into the components of the glass prisms 363, 364 again through the quarter wave plate 367, each of the 2 lights is further split into 2 lights by the

same principle as that described above, which allows 4

light-fluxes to be formed in total. The adjustment of

heights of the trapezoid-shaped glass prisms makes it

possible to arbitrarily set a spacing between the

5   light-fluxes, thereby making it easy to obtain the

light-fluxes with the spacing of 0.4 mm, i.e., the

light-fluxes that are exceedingly proximate to each

other. In the drawing, there is illustrated the case

where the number of emitted light-fluxes is 32 with

10   reference to 8 incident light-fluxes. Incidentally, it

is also allowable to locate a pinhole plate in

proximity to the quarter wave plate 367.

FIG. 56 is a diagram for illustrating the

configuration of another embodiment of the multi light-

15   source distribution unit 11f. By using prisms 500a,

500b, ⋯ illustrated in the drawing which have a

polarization beam splitter and a total reflection

mirror, it becomes possible to split a beam into 2

beams. The use of the prisms by the number of 8 allows

20   lights from 8 laser light-sources to be split into 16

lights. Incidentally, it is also allowable to employ,

as a portion of the polarization beam splitter, a half

mirror-shaped beam splitter where the transmittance =

the reflectance (= an order of 50%).

25   FIG. 57 is a diagram for illustrating the

configuration of another embodiment of the multi light-

source distribution unit 11f. The prisms 500a, 500b, ⋯

in FIG. 56 are arranged, and further thereon, there is

used a prism 510 formed by accumulating larger-sized prisms in sequence. The half mirror-shaped beam splitters split a laser light. Repeating this at 4 stages allows 1 laser light to be split into 16 laser

5   lights.

In the multi light-source distribution units of the configurations in FIGS. 56, 57, there exists a limit to a task of making the prism sizes smaller. Although the spacing between the laser lights to be

10 split is allowable down to an order of 1 mm, it is getting gradually difficult to make the spacing even narrower. On account of this, in such a case, the combined use of the distribution units with the multi light-source distribution unit in FIG. 54 or FIG. 55

15 permits the laser lights to be split into the light-fluxes with a more microscopic spacing.

In general, like the configuration of an embodiment of the multi light-source distribution unit illustrated in FIG. 67, a plurality of multi light-

20 source distribution units 550, 551 are combined with each other. This makes it possible to construct another multi light-source distribution unit. Namely, the multi light-source distribution units in FIGS. 52 to 57 and what is more, multi light-source distribution

25 units up to in FIGS. 68, 69 explained later are combined, thereby making it possible to construct another multi light-source distribution unit. Inciden-tally, the number of the laser light-sources is

selected suitably in accordance with the number of the
splitting, the optical output of the laser light-
source, and the light intensities of the excitation
spots needed on the surface of the substrate 2f.

5   FIG. 68 is a diagram for illustrating the
configuration of another embodiment of the multi light-
source distribution unit 11f. A laser light emitted
from a laser light-source 2000 is caused to pass
through a lens 2001, and is scanned by a beam scanning
10 unit 2002 such as a galvanometer mirror and a polygon
mirror, then being launched into end surfaces of
optical fibers 3001, 3002, ⋯ the end surfaces of which
are located at a focal point position of the lens 2001
in an arc-like configuration. The other end surfaces
15 of the optical fibers 3001, 3002, ⋯ are located with a
fixed spacing. This allows the laser lights to be
split into a large number of light-fluxes, thereby
making it possible to implement the configuration in
the present embodiment.

20   FIG. 69 is a diagram for illustrating the
configuration of another embodiment of the multi light-
source distribution unit 11f. The laser light emitted
from the laser light-source 2000 is scanned by a beam
scanning unit 2003 such as the galvanometer mirror and
25 the polygon mirror, and is caused to pass through a
lens 2004, then being launched into the end surfaces of
the optical fibers 3001, 3002, ⋯ that are arranged in a
line. The respective split laser lights can be

extracted from the other end surfaces of the optical fibers, which makes it possible to implement the configuration in the present embodiment.

Next, referring to FIGS. 58 to 63, the
5 explanation will be given below concerning the configu-rations of embodiments of the multi-detection unit 17f. Like the present example, images of the excitation spots, the size of which is 2 μm in diameter and between which the spacing is 20 μm, are image-formed
10 with an appropriate magnification. A pitch of detectors determines the magnification. The detectors 600, 601, ⋯, such as photomultiplier tubes and photo-diodes, are arranged in agreement with the image-formed positions (FIGS. 58, 59). Otherwise, the detection is
15 executed using a censor 602 such as a photodiode line censor, a multi-anode type photomultiplier tube, and a CCD line censor (FIGS. 60, 61). In the case of the censor 602, a spacing between the individual detectors can be made narrower in comparison with the spacing in
20 the methods in FIG. 58 and so on. For example, in the multi-anode type photomultiplier tube, the optical system is adjusted so that a spacing between the individual anodes is substantially equal to 1 mm and 50 times image-forming magnification is accomplished.
25 Incidentally, through the use of pinholes 610 in FIGS. 58, 59, and 60, the multi-detection unit becomes a confocal point detecting system, thereby enhancing a position resolution and a depth resolution and improv-

ing the accuracy of the fluorescent light measurement. In the case of using the photodiode line censor and the CCD line censor, it is also allowable to cause the images to be image-formed at arbitrary positions and to

5 detect signal intensities of the pixels at the image-formed positions. It is also possible to obtain the same effect as that of the pinholes by precluding signals from the pixels at portions other than the excitation light spots. It is also possible to execute

10 the detection through optical fibers 620, 621 (FIGS. 62, 63).

FIG. 64 is a diagram for illustrating an example of the images detected using the present apparatus. The drawing illustrates the fluorescent

15 light spot images captured on the slide glass. The spots coupled with the fluorescent marker-attached DNAs by the above-described method are formed on the slide glass. The size of the respective spots are about 200 μm, and the respective spots are formed on a lattice

20 with a spacing of about 0.4 mm. The entire area over which the spots are formed is about 12 mm long and wide, and FIG. 64 indicates a portion thereof. In the drawing, the respective spots are increased in their specimen concentration in sequence from the above. As

25 a result, the fluorescent light intensity has been detected in such a manner as to be increased in sequence from the above to the below.

Next, the explanation will be given below

concerning the data detection and an image reconstruc-
tion (i.e., an operation of the data processing unit).
In order to detect the signals over the area of 12 mm
square in the unit of 2 μm, the area of 12 mm square is

5   divided into 6000×6000 areas. Let these areas be
represented by a reference notation: area (i, j),
where i (= 0 - 5999) denotes the positions in the x
direction and j (= 0 - 5999) denotes the positions in
the y direction. The detection of the fluorescent

10   light intensities based on the scanning by the irradia-
tion spots is executed in the same way as was explained
in FIG. 65. Explaining the scanning performed by the
respective irradiation spots (S0, S1, ···, S63), with
respect to the scanning by the irradiation spot S0,

15   area (0, 0) is first irradiated with the irradiation
spot S0 and then, following the scanning in the y
direction, the irradiation spot scans the areas from
area (0, 1) to area (0, 5999). With respect to the
scanning by the irradiation spot S1 as well, area (10,

20   0) is first irradiated with the irradiation spot S1 and
then, following the scanning in the y direction, the
irradiation spot scans the areas from area (10, 1) to
area (10, 5999). The other irradiation spots S3 to S63
perform the scanning in much the same way. With

25   respect to the scanning by the irradiation spot S63 as
well, area (630, 0) is first irradiated with the
irradiation spot S63 and then, following the scanning
in the y direction, the irradiation spot scans the

areas from area (630, 1) to area (630, 5999).

At that time, the signals from the optical detector corresponding to each spot are sampled every time each spot passes through the areas, then being

5 A/D-converted. The signals are expressed as sig (k, j), where k corresponds to a position number (0 - 63) of the spots and j takes the values of 0 to 5999 at the positions in the y direction. Namely, the signals from the spot S1 are expressed as sig (1, j), j = 0 - 5999.

10 These signals are stored into image storing memories corresponding to the areas of area (i, j). Letting the image storing memories be represented by image (i, j) where i (= 0 - 5999) and j (= 0 - 5999) denote the positions in the x, y directions, respectively, the

15 signals obtained by the above-described scanning at one time are stored into

image(k×10+X,j) = sig(k,j)(X=0, k=0-63, j=0-5999).

Incidentally, X denotes an X position of the spot S0 (In this case, X = 0).

20 Then, as illustrated in FIG. 65, the Y position is returned back to 0, and the X position is displaced by the amount of $+2\mu$m (X = 1), then executing the detection in the same way. The signals obtained are stored into

25 image(k×10+1,j) = sig(k,j)(k=0-63, j=0-5999).

Repeating this operation ten times causes areas indicated by i = 0 - 639, j = 0 - 5999 in image (i, j) to be replaced by the measured signals.

Next, the X position is displaced by + 1260 μm (a position of X = 640), then, similarly, causing the above-described operation to be performed and storing the obtained signals into

5 image(k×10+X,j) = sig(k,j)(X=640-649, k=0-63, j=0-5999). This operation causes areas indicated by i = 640 - 1279, j = 0 - 5999 in image (i, j) to be replaced by the measured signals. Moreover, the X position is displaced by +1260 μm (a position of X = 1280), then

10 repeating the above-described measurement. These are repeated, thereby displaying the X position up to a position of X = 5760, and causing the scanning to be performed 10 times further. This measures all the areas indicated by i = 0 - 6399, j = 0 - 5999 in image

15 (i, j) (Although this area is wider than area (i, j), it is all right to extract a needed area alone). This image (i, j) is then converted into a picture, which is displayed on the monitor or the like.

In the present embodiment, there are provided

20 the excitation spots and the optical detecting unit corresponding to each spot. There occurs no problem if the intensities of all the excitation spots and a sensitivity characteristic of the optical detecting unit coincide with each other. If not, there appears a

25 stripe-shaped pattern in the obtained picture. In such a case, there is a need of a mechanism for correcting the sensitivity characteristic for each spot. Thus, in FIG. 51, there is provided an intensity correction

value storing unit 22f. In order to make the correc-
tion, data for the amendment is created first.
Preparing a specimen having equal fluorescent light
intensities (or locating light-sources that emit lights

5   equally at the respective spot positions), the inten-
sities at that time are measured for each spot. In
addition, the intensities at the time when the lights
are light-shielded are also measured for each spot. In
this way, back (k), i.e., a background value on the

10 per-spot measurement basis, and corr (k) are stored
into the intensity correction value storing unit 22f.
Here, corr (k)(k is the position number (0 - 63) of the
spots) are the reciprocal values of the signal
intensity values at the time when a light intensity

15 becoming a reference value is detected (the signal
intensity values obtained by subtracting the background
value). By outputting signal values sig (k, j)
corrected as

$$sig(k, j) = (sig(k, j) - back(k))corr(k)$$

20 toward the measured signals sig (k, j), it becomes
possible to correct a variation in the sensitivity
among the spots.

In contrast to the circumstances that the
ordinary apparatus uses only 1 fluorescent-light

25 excitation irradiation spot, the present invention
allows the substrate to be simultaneously irradiated
with the plurality of (64, for example) fluorescent-
light excitation irradiation spots. This condition

results in the following effects:

When detecting the entire area in a fixed time-period, it is possible to lengthen the irradiating time per irradiation spot (64 times in the present

5   embodiment). This enhances the fluorescent-light detecting sensitivity. Also, lengthening the irradiating time per irradiation spot also makes it possible to slow the displacement speed of the stage. This enhances the mechanical stability and durability as

10   well. Also, equating the irradiating time per point makes it possible to shorten a time needed to measure the whole of a predetermined region on the substrate. This allows the high-speed characteristic to be implemented. In particular, when enhancing the detec-

15   tion resolution, namely, when making smaller the size of the irradiation spots on the substrate, if a target that has been measured conventionally with a resolution of, for example, 10 μm is measured with a resolution of 1 to 2μm, it is required to measure in sequence the

20   irradiation spot regions that are 100 to 25 times more in number. On the other hand, just like the present invention, by simultaneously irradiating the substrate with the plurality of (64) fluorescent-light excitation irradiation spots so as to execute the fluorescent

25   light detection, it becomes possible to accomplish the high-resolution and high-speed characteristics.

Incidentally, when performing the irradiation with the split laser lights, the intensity of the

individual split laser lights becomes weaker by the amount of being split. The use of a larger output laser apparatus, however, allows this problem to be solved easily.

5   According to the present invention, it becomes possible to measure the DNA probe array and so on with a high-resolution, at a high-speed, and with a high-sensitivity.

FIG. 70 is another embodiment-illustrating

10 diagram in the present invention. Laser light-sources 211g, 212g, 213g ··· ··· emit parallel round beams that are converted from diffused lights emitted from semiconductor lasers having a wavelength of 635 nm. The K light beams emitted from the respective laser light-

15 sources are launched into multi-beam splitters 22g with a pitch P. The multi-beam splitters 22g are arranged with a pitch P by the number of K, and split each of the incident beams into 2 beams, thereby forming 2K beams with a pitch P/2 and in parallel to each other.

20 These beams have been formed into elliptically polarized lights, linearly polarized lights, or circularly polarized lights, the elliptically polarized lights having the longer axis in the directions that forms ±45 degrees toward y, z axes of the illustrated

25 coordinate.

The 2K beams are arranged with the pitch P/2, then being launched into a multi lens 231g. The multi lens 231g is pasted on a prism 232g. The beams that

have passed through the multi lens pass through the
prism 232g, then being launched into multi pinholes
positioned at the boundary with a prism 233g.

     At both of the bases of the prisms 232g,

5 233g, trapezoid-shaped glasses that differ from each
other slightly in thickness are bonded to each other.
The bonded plane is formed as a polarization beam split
plane. As a consequence, the beams having passed
through the prism 232g are converged into the respec-

10 tive pinholes of a pinhole array 234g having the 4K
pinholes with a pitch P/4. The pinhole array plays a
role of eliminating a stray light so as to irradiate
the DNA chip with the multi spots having a less noise,
the stray light existing in a portion other than the

15 pinhole unit and becoming the noise.

     At the time of passing through the above-
described prism 232g, the lights having passed through
the polarization beam split plane become P-polarized
lights (linearly polarized lights perpendicular to the

20 z axis), and the lights reflected by the beam split
plane become S-polarized lights (linearly polarized
lights in the z axis). On account of this, the half of
the lights having passed through the multi spots are
the P-polarized lights, and the remaining half are the

25 S-polarized lights.

     Directly behind the pinhole array, there is
located a half wave plate or a quarter wave plate. The
half wave plate has an optical axis inclined at 22.5

degrees toward the above-described P-polarized and the
S-polarized lights. Also, the quarter wave plate has
an optical axis inclined at 45 degrees toward the
lights. The P-polarized and the S-polarized lights
5   that have passed through such a wavelength plate after
passing through the pinholes become linearly polarized
lights inclined at ±45 degrees toward the z axis in the
case of the half wave plate, and become right-handed
and left-handed circularly polarized lights in the case
10   of the quarter wave plate.

     The lights having passed through the above-
described wave plate 235g are launched into the above-
described trapezoid-pasted prism 233g (Additionally,
the difference in thickness between the trapezoid-
15   shaped prisms is one-half of the thickness of the
above-described trapezoid-pasted prism 232g). The
pasted plane, i.e., the trapezoid-shaped base of the
prism, is formed as the polarization beam split plane.
As a consequence, the 4K lights having passed through
20   the pinhole array and the wavelength plate are
increased by 2 times in number even further, eventually
having a pitch of P/8 and becoming 8K in number. On
account of this, the lights are emitted from the prism
233g as if 8K point light sources (secondary point
25   light sources) existed at the position of the pinhole
array 234g.

     The lights from the 8K secondary point light
sources formed in this way pass through a lens 24g, a

mirror 25g, a light amount adjuster 26g, a wavelength selection beam splitter 30g, and a high NA objective lens 3g, then image-forming images of the 8K point light sources onto a detection plane 51g of a DNA chip

5   5g. Incidentally, the light amount adjuster 26g is provided in order to adjust a light amount of the fluorescent-light detection excitation lights depending on to what extent the fluorescent material exists on the detection plane 51g. Namely, if the concentration

10 of the fluorescent material is high all over the entire DNA chip or fluorescent light detection sample, a driving source 260g is driven so that a ND filter 262g of, for example, 10% is inserted into the excitation light optical path. However, changing the intensities of all

15 the excitation light beams in this way, in some cases, results in narrowing the dynamic range of the detection. Consequently, in such a case, it is possible to adjust the intensities of the individual excitation light multi spots using a light modulator array not

20 illustrated.

Also, when it is required to execute the detection in a dynamic range of, for example, 2^N, the following detecting method is also possible: Setting a ND filter 261g to be 100% (i.e., there exists no ND

25 filter) and setting the ND filter 262g to be a transmittance of $100 \times 2^{N/2}$ %, the ND filters are exchanged so as to execute a 2-picture detection toward the entire picture. Then, both of the detected

pictures are superimposed, thereby detecting the fluorescent light images in the dynamic range of 2^N. In this way, the detection of the 2 pictures necessitates a time twice as long as the time in the ordinary

5   method. The present invention using the multi spots, however, makes it possible to execute the detection at a speed that is more than 5 times higher as compared with the speed in the conventional methods.

The 8K point light source images that have

10 illuminated the detection plane of the DNA chip illuminate and excite a target DNA having the fluorescent material at a tip hybridized with a probe DNA.

FIG. 71 is a diagram for indicating the relationship between the multi spots and the details of

15 an area 50g where the target DNA exists on the glass 51g of the DNA chip 5. A numeral 505 denotes a cell. The cells are arranged in the x, y directions with a fixed pitch. In each cell, a desired DNA fragment, which is also referred to as the probe DNA, is attached

20 to the glass for each address in the arrangement of the cells. This probe DNA is a DNA that usually includes the same nucleotide sequence in each cell, and a different DNA is usually probed in a different cell. A sample solution of the target DNA obtained by attaching

25 the fluorescent material to ends of plural types of DNA fragments formed by collecting, refining and amplifying a target to be inspected, i.e., a specimen of a creature, is poured onto the DNA chip prepared in the

above-described manner, thereby causing the hybridiza-
tion to occur. Namely, a target DNA corresponding to
the nucleotide sequence of the probe DNA in each cell
is coupled with the probe DNA, namely, is hybridized

5 therewith. The DNA chip in FIG. 71 has been formed in
the above-explained manner.

With reference to the cells 505, the multi
spots 2001g, 2002g, 2003g, $\cdots$ $\cdots$, 20Mg, i.e., the
pumping lights, are arranged with a pitch that is an

10 integer multiple of the cell pitch. In the case of
FIG. 71, this integer is equal to 2. The beam diameter
of the multi spots is equal to substantially Δ, and the
pitch between the multi spots is equal to $N\Delta$. In the
case of FIG. 71, $N = 10$ and the detection is executed

15 by dividing one cell into 5×5 pixels. An object of
such a division is as follows: When a foreign
substance or the like is attached in a cell, the
foreign substance generates a strong fluorescent light,
thereby causing an error in the detected data. Accord-

20 ingly, judging that a fluorescent light exhibiting a
detection intensity larger than the strong fluorescent
light detection intensity is generated by the foreign
substance, an average fluorescent light intensity
within the cell is determined by excluding the pixels

25 where the foreign substance has been attached.

The DNA chip is scanned in the x direction as
is indicated by A in FIG. 71, and when the multi spots
scan the cells positioned at the right end, the multi

spots are displaced by the amount of one pixel Δ in the y direction, and the scanning is executed in the x direction again. This is repeated, and when the scanning of N pixels (in the drawing, 10 pixels) is over,

5 the multi spots are displaced by the amount of $MN\Delta$ in the y direction, and the above-described operation is repeated, thereby scanning all the cells. FIG. 72 is a diagram for illustrating the above-described operation. Letting the transverse axis represent time, the longi-

10 tudinal axis in an upper graph indicates the displacement quantity of the y stage, and the longitudinal axis in a lower graph indicates the displacement quantity of the x stage. On the x stage, the scanning for the detection has been executed during a time t_s, and the x

15 stage is returned back to the initial position during a time $t_t - t_s$. The detection has been executed in the forward-step path alone. When the accuracy of the stage is sufficiently high, the detection is executable in the backward-step path as well.

20 As illustrated in FIG. 70, the simultaneous detection of the fluorescent lights by the amount of the M pixels is executed in the following way: The fluorescent lights generated by the irradiation with the excitation multi-spot lights are received by the

25 objective lens 3g. Out of the transmittance fluorescent lights having passed through the objective lens, the fluorescent lights at the wavelength in proximity to 670 nm are reflected by the wavelength

selection beam splitter 30g. Then, the reflected fluorescent lights are guided into a detecting optical system. Namely, the reflected fluorescent lights pass through a mirror or a wavelength selection beam

5 splitter, i.e., 32g, 34g, 35g, and 38g, and pass through an image-forming lens 33g, then being image-formed at light-receiving apertures of multi-channel photomultiplier tubes 101g, 102g. At the respective light-receiving apertures, there is provided a light-

10 shielding plate where pinholes are bored, the diameter of the pinholes being substantially equal to that of the images of the excitation multi-spot lights. Only the fluorescent lights that have passed through the pinholes are detected, thereby executing the confocal

15 point detection.

An upper graph in FIG. 73 is a graph obtained by enlarging the time axis of the lower graph in FIG. 72. In the drawing, the time t_s, which is needed to execute one scanning, is seen in more detail. In a

20 lower graph in FIG. 73, although the transverse axis, i.e., the time axis, is identical to the transverse axis of the upper graph in FIG. 73, the longitudinal axis indicates a timing of a time of the photon counting performed by a weak light detector such as the

25 photomultiplier tube. This graph means that the photon counting is performed during a time for which the signal level is 1 and the photon counting is not performed during a time for which the signal level is

0. During the one-sectioned time for which the signal level is 1, the signal by the amount of one pixel is detected. After the signal level becomes 0, when the signal level 1 starts next, the next pixel is detected.

5 Since there exist L_x pixels in one scanning in the x direction, the detecting time per pixel becomes equal to t_s/L_x or less. The time needed to perform the photon counting on the per-pixel basis is required to correspond to the position of the cell in the DNA chip.

10 Consequently, the signal for the starting time of the photon counting is created based on a signal from the length-measuring device for measuring the x stage's position.

If the finishing point-in-time of the per-

15 pixel photon counting is determined in accordance with the information from the stage's position length-measuring device, when the stage is not displaced uniformly, the per-pixel photon counting time is varied for each pixel. This requires that the finishing time

20 be a time that has elapsed by a fixed time after the starting time. For the purpose of this, pulses of a high-frequency (frequency νr) pulse generator is counted. The counting is started from the starting of the photon counting, and the photon counting is

25 finished at the time when the pulse number counted reaches a fixed pulse number Nr. Namely, with reference to whatever pixel, the photon counting is executed during a time of $Nr/\nu r$. A control apparatus

1g in FIG. 70 is a system for controlling the above-described operation. A reference numeral 12g denotes a PC (personal computer), which performs the control of all the operations. A PC 13g generates a controlling

5   and driving signal of the above-described stage 4g, and fetches the position information of the stage 4g from the length-measuring device.

In the above-described manner, the fluorescent lights from the 64 pixels in the x direction

10 are detected simultaneously and, through the scanning by the stage, in sequence. Photon counting signals S_{PM} to be obtained from each pixel are detected in the manner as is illustrated in FIG. 74. Namely, focusing an attention on one photomultiplier tube out of the

15 multi-channel photomultiplier tubes, in the case of weak detected fluorescent lights, photomul signals are obtained as photon detection pulse signals the pulse time-width of which is equal to about 30 ns with a low frequency as are illustrated in an upper graph in FIG.

20 74. Then, pulse signals with a fixed width are generated from the rising edges of the photomul signals. Otherwise, in the case where the photomul signals exceed a fixed signal level I_T as are illustrated in the upper graph in FIG. 74, the photomul

25 signals are converted by a comparator circuit into fixed level signals just like the signals S_{PC} illustrated in a lower graph in FIG. 74. The pulse-shaped signals formed in this manner are counted using a

counter. This allows the detected photon number to be determined within the detecting time $t_s/L_x - \Delta t = Nr/\nu r$.

FIGS. 75A to 75D illustrate the case where the detected fluorescent light intensities are high.

5 The drawings illustrate the photomul detection signals S_{PM} and the signals S_{PC} after being comparated. The photon pulses are detected with a high frequency and accordingly, in some places, 2 or more of the pulses have been detected in a state of being superimposed on

10 each other. On account of this, among the signals after being comparated, there appear signals having larger width. S_{PC}' is a diagram resulting from enlarging a time of the signals S_{PC} between a point-in-time A and a point-in-time B. If the detection signals have been

15 detected in the state of being superimposed between a signal and a signal subsequent thereto, the pulse-widths of the signals after being comparated are increased. As a result, calculating simply the pulse number of the signals after being comparated results in

20 an increase in the error. Accordingly, as illustrated in a graph in FIG. 75D, constant high-frequency pulse signals are counted with S_{PC}' employed as the gate. The use of the counted signals S_{PWC} thus obtained makes it possible to determine the photon number more precisely

25 than counting the pulses of the S_{PC} signals. Moreover, by correcting the counted value of the S_{PWC} signals with a downward-convex univalent function $F(S_{PWC})$, it becomes possible to execute even more precise photon counting.

The above-described method, however, is insufficient to implement the even broader dynamic range requested in the DNA inspection. Namely, in the DNA inspection, the dynamic range of several photon

5 pulses to one hundred and tens of thousands of photon pulses is requested within the time needed to detect one pixel. Nevertheless, since the width of the photon detection pulses is equal to tens of ns, when trying to implement the entire dynamic range by the photon count-

10 ing, tens of ns X one hundred and tens of thousands = several m sec become required as the per-pixel detecting time.

Accordingly, even if the number of the multi-spot excitation lights is equal to 64, when the number

15 of the pixels is 6000X6000, it turns out that it takes 30 minutes or so to execute the detection.

FIG. 76 illustrates an embodiment for solving this problem. The output signal of the multi-channel photomultiplier tube 101g is inputted into an analogue

20 switch 1A. A PC 10g connects an initial condition of the analogue switch with B, i.e., a photon counting circuit 1B. This means that, when executing the fluorescent light detection on the per-pixel basis, the photon counting is executed first. FIGS. 77A, 77B,

25 which illustrate the time-elapsing change of the photon counting signals, correspond to the case where the fluorescent lights are exceedingly weak and the fluo-rescent light intensities are detected by the photon

counting detection.

FIG. 77A illustrates the pulse signals S_{PC} formed by binarizing the photon pulse signals with the use of the comparator, the photon pulse signals having been detected by the photomultiplier tubes. FIG. 77B illustrates a counted signal NS_{PC} obtained by counting the pulse number of the pulse signals in FIG. 77A. If the count signal NS_{PC} does not exceed a determined threshold value N_0 at a point-in-time at which a time α (t_s/L_x) has elapsed after the starting of the counting, the analogue switch continues the photon counting in the present state that will remain unchanged, then executing the photon counting during the per-pixel fluorescent light detecting time $t_s/L_x - \Delta t$. With respect to α in the time $\alpha(t_s/L_x)$ at which this judgement should be made after the starting of the counting, it is all right to assume α to be substantially 0.01 to 0.3. Namely, this time is satisfying enough if it is of an order of a time during which the photon counting pulses are at the maximum and approximately 10 or more pulses are detected. The above-described phrase "at the maximum" means the case where the pulse signals have been generated at the highest ultra-density within this time.

FIGS. 77C to 77E illustrate the case where the fluorescent light intensities become higher and executing the photon counting causes inconveniences to occur, namely, the case where 2 or more of the photon

counting pulses are superimposed on each other at the same time or partly. In this case, the after-comparator pulse signals S_{PC}, when formed by comparating the partly superimposed pulses, are increased in their

5 widths as illustrated in FIG. 77C. FIG. 77D illustrates a counted signal NS_{PC} of the pulse signals in FIG. 77C. The counted signal NS_{PC} exceeds the threshold value N_0 at the point-in-time $\alpha(t_s/L_x)$, which shows that the fluorescent light detection by the photon counting

10 is not suitable for the fluorescent light intensities at the point-in-time. Thus, at this point-in-time, the analogue switch in FIG. 76C is connected to C so as to integrate the photomul signals in an analogue manner by an integrating circuit 1C. In this way, by judging, on

15 the per-pixel basis, whether the fluorescent light detection should be executed by the photon counting or the analogue integration, the detection is executed in such a manner that the optimum method is selected for each detection.

20 Incidentally, although not illustrated, if the fluorescent light intensities become higher than those in the state in FIG. 77C, the pulses in FIG. 77C are connected with each other and as a result, the pulse-counted value become equal to several or 0. At

25 this time, as illustrated in FIG. 75D, employing the signals S_{PWC} for counting the signals S_{PC} with the use of the rectangle-shaped high-frequency pulses with the constant frequency, a pulse-counted signal NS_{PWC} thereof

is given as is illustrated in FIG. 77E. The use of the
signal NS_{PWC} makes it possible to judge that the value of
the signal exceeds a threshold value N_1. Then, switch-
ing the analogue switch to C allows the analogue

5 detection to be executed without a mistake even in the
case of the detection of the even higher fluorescent
light intensities.

Once the switching to the analogue detection
has been performed, the circuit 1C starts the analogue

10 integration with the switching point-in-time employed
as the starting point-in-time. As illustrated in FIG.
78A, setting a target pixel detection starting point-
in-time to be the starting point-in-time (this point-
in-time is assumed to be 0), the circuit 1C starts the

15 integration from the point-in-time $\alpha(t_s/L_x)$. Then, as
is the case with the photon counting, the circuit 1C
continues the integration until the point-in-time t_s/L_x
$- \Delta t$, and A/D-converts the integrated value at this
point-in-time. The A/D-converted digital information

20 IC01 is transferred to a circuit 100g. In the case
where the fluorescent light intensities are weak, the
photon counting is continued, and the photon counting
signals ranging from the point-in-time 0 to the point-
in-time $t_s/L_x - \Delta t$ are counted. The counted digital

25 information IB01 is also transferred to the circuit
100g. The results of the photon counting digital
information and the analogue integration digital infor-
mation, which have been transferred to the circuit 100g

in the parallel manner as described above from the
respective channels of the multi-channel photo-
multiplier tube 101g, are transferred to the PC 11g,
i.e., the personal computer. As having been described

5   previously, it was recognized at the point-in-time α
(t_s/L_x) whether the detection executed in each channel
has been executed by the photon counting or the
analogue integration. Consequently, the PC 11g selects
and employs the method suitable for the detection for

10 each channel. Namely, the selection signals therefor
are simultaneously transferred to the information IC01,
IC02, ⋯ ⋯ in Fig. 76.

      Incidentally, in the above-described embodi-
ment, it was judged at the point-in-time $\alpha(t_s/L_x)$ which

15 method should be selected of the photon counting and
the analogue integration. However, it is also
allowable to execute the detection from the beginning
by both of the methods simultaneously and to perform
the selection after the time $t_s/L_x - \Delta t$ has elapsed.

20   FIG. 78A indicates a variation in the
integration signal when the analogue integration
detection is executed. Between the point-in-time α
(t_s/L_x) at which the analogue integration detection was
started and the point-in-time $t_s/L_x - \Delta t$, the integrated

25 value has been increasing with an inclination propor-
tional to the magnitudes of the detected fluorescent
light intensities. Then, the integrated value at the
point-in-time $t_s/L_x - \Delta t$ undergoes the sample holding,

then being A/D-converted. The analogue detection as described above makes it possible to capture the variation in the signal intensities over a dynamic range of approximately 2 order.

5   Next, referring to FIG. 78B, the explanation will be given below concerning a method of making the detection executable in the range of the detection of the even higher fluorescent light intensities. In this case, since the integrated value had been saturated

10 before the analogue integration detection reached the point-in-time $t_s/L_x - \Delta t$, the detection at this point-in-time is impossible: However, using the high-frequency pulse signals S_{hc} used in FIG. 75D, by counting $(t_F - \alpha(t_s/L_x))$, i.e., a time ranging from the

15 point-in-time $\alpha(t_s/L_x)$ at which the integration was started to a point-in-time t_F at which the integration was saturated, the detected intensity I is determined by the following equation since the integration-saturated value I_F had been known in advance:

20        $$I = I_F \cdot ((1-\alpha)t_s/L_x - \Delta t)/(t_F - \alpha(t_s/L_x))$$

In this way, measuring the time from the integration-starting point-in-time to the integration-saturated point-in-time allows the dynamic range to be enlarged by approximately 1 order further.

25   In order to count the saturated point-in-time as described above, it is well enough to add the

following function to the analogue integrating circuit
illustrated in FIG. 76. Namely, setting the integra-
tion signal of the integrating circuit 1C to be the 1st
input and setting a signal level equivalent to the
5   predetermined integration-saturated value to be the 2nd
input, these two inputs are inputted into the
comparator circuit. Then, from variations in the
outputted signals resulting from the inputs, it can be
assumed that the counting has been finished. This
10   allows the saturated point-in-time to be counted.

As having been explained so far, the fluo-
rescent lights detected by the multi-channel photo-
multiplier tubes 101g, 102g are detected in the 64
channels in parallel, and are inputted into the circuit
15   100g in parallel and are converted into the time-
sequential signals, then being transferred to the PC
11g. By scanning the x stage in FIG. 70, as illus-
trated in FIG. 71, the 64 pixels arranged in the y
direction are detected simultaneously and, in the x
20   direction, in sequence. Although not illustrated, the
optical length-measuring device is attached to each of
the xy stage 4g in FIG. 70, and the length-measuring
device measures the displacement quantities of the xy
stage. The dimensions Δx, Δy of the fluorescent light
25   detected pixels on the DNA chip are each assumed to be,
for example, 2 μm. The measurement of each pixel is
started from a point-in-time at which the length-
measuring device of the x stage measures the displace-

ment of 2 μm.

Hereinafter, the explanation will be given concerning a concrete embodiment of the multi-spot fluorescent light detection or the multi-spot DNA inspection according to the present invention. The scanning operation is performed in the x direction, and the shifting operation by the amount of 1 pixel on the basis of one scanning in the x direction is performed in the y direction, and the x positions are shifted by a large amount every 10 scannings. This is repeated, thereby executing the detection or the inspection in the 2-dimensional manner.

When a stage's position length-measuring signal of the measurement starting is detected by the scanning in the x direction, the photon counting is started first. It is assumed that L_x, i.e., the detected pixel number in the x direction, is equal to 6400, and the pixel pitch is equal to 2 μm, and t_s, i.e., the time needed to scan this 2 μm, is equal to 60 μsec. The width of the pulses generated after the photomultiplier tube has received 1 photon is equal to substantially 30 n sec. Consequently, when executing the photon counting during 40 μsec out of the time of 60 μsec during which the spot has passed through one pixel, it becomes possible to count hundreds to thousands of photons using the photon counting method. Toward the lights the intensities of which are higher than this, the pulse signals are connected with each

other and accordingly the analogue integration detection is executed. The dynamic range of this analogue detection is equal to approximately 100. Consequently, by using the photon counting and the analogue integra-

5 tion together in a manner of being switched to each other by the above-described method, it becomes possible to obtain the dynamic range of approximately 10000.

Moreover, by using together the method

10 explained in FIG. 78B by which the detected intensity is determined from the point-in-time at which the analogue integration was saturated, it becomes possible to enlarge the dynamic range by 1 order further. As a consequence, it becomes possible to execute the fluo-

15 rescent light detection in a short time over a broad dynamic range of 2^{16}, i.e., 65536, namely, from the weak detected lights of several photons in 40 μsec up to the strong detected lights attaining to 100000 photons.

When the above-explained fluorescent light

20 intensities include 3 areas, i.e., the photon counting area, the analogue integration area, and an analogue integration-saturated time detecting area, in order to cause the respective boundaries therebetween to be connected with each other smoothly, the calibration is

25 performed in advance using strong detected lights corresponding to the boundary regions.

Although, in the above-described embodiment in FIG. 76, the detection is executed by time-dividing

the photon counting and the analogue integration, it is also possible to use both of them all the time and to select the signal to be employed in correspondence with the intensities of the detected fluorescent lights.

5   The above-described switching between the detecting methods is not limited to the fluorescent light detection, but is generally effective in the case where the per-pixel detecting time Tp is restricted to a short time. Namely, when Nr, i.e., the ratio between

10   Tp and the time-width Δt of the photon detection pulses, is smaller as compared with the requested detection dynamic range Nd, namely, when

$$Tp \;/\; \Delta t = Nr < Nd$$

holds, the detection becomes executable in the broad

15   range extending from the weak photon counting area up to the strong lights the photon counting of which is impossible.

The scanning by the amount of 6000 pixels is performed in the x direction at a speed of 60 μsec/1

20   pixel and at this time, in the y direction, 64 pixels are detected simultaneously with 10 pixels skipped. Accordingly, at a point-in-time at which the one scanning is over in spending 360 msec, 384000 pixels have been detected in the above-described broad dynamic

25   range. When the one scanning is over, the stage is displaced in the y direction by the amount of 1 pixel,

i.e., 2 μm, then detecting the adjacent pixel line similarly. This operation is repeated 10 times, thereby executing the detection of 640×6000 pixels. Even if accelerating and decelerating times needed to return

5 the stage are added, the detection of the pixels in the above-described number is finished in spending 4 to 5 seconds.

Next, the stage is displaced largely in the y direction by the amount of 640 pixels, i.e., 2 μm ×

10 640 = 1.28 mm, then repeating the above-described operation. Repeating this large displacement 10 times allows 6400×6000 pixels to be detected in spending substantially 40 to 50 seconds. Namely, it becomes possible to accomplish the fluorescent light detection

15 or the weak light detection of the images of 6000×6000 pixels the minimum detection intensity of which is of several photons with a resolution of 2 μm, in the dynamic range of 2^{16}, and within 1 minute.

The above-described embodiment is of the case

20 where the detecting time requested for each pixel is short. However, in the case where the per-pixel detecting time is larger by 1 order or more and the dynamic range of 2^{16} is required similarly, the method illustrated in FIG. 78B where the integration-saturated

25 time is determined becomes unnecessary. Also, if the width of the photon detection pulses from the photomultiplier tube can be shortened even further, the photon counting alone or the combination of the photon

counting and the analogue integration detection makes the detection possible.

When trying to apply the DNA inspection, in particular, the DNA inspection using the DNA chip, to a usage such as group checkup where a large number of samples are dealt with, it becomes important to implement an inspecting apparatus having the character- istics of a high-sensitivity, a high-resolution, a broad dynamic range, and a high-speed. This implemen- tation has been made possible by the following method: Using the multi-spot excitation lights or the sheet- shaped excitation beams explained in the previously- mentioned embodiments, the fluorescent lights from a plurality of pixels are detected simultaneously, and the relative positions between the excitation lights and a sample are changed by the scanning so as to detect the fluorescent lights in sequence.

Detecting the 64 (M = 64) pixels simultane- ously has allowed the 6000×6000 pixels to be detected in spending a time less than (300 μsec/pixel), namely, the average pixel detecting time has become shorter than (300 μsec/M). As a result, it has become possible to execute the detection in a time less than 3 minutes per sample. This result can respond to and satisfy the needs of the high-speed inspection because, conven- tionally, it took 10 minutes or more to detect the fluorescent light images of the pixels in the above- described number. Furthermore, by using the photon

counting and the analogue integration together, it
becomes possible to execute the detection in a time
less than (50 μsec/pixel), thereby eventually allowing
the detection to be executed within 1 minute.

5   Moreover, in the above-described fluorescent
light detection using the multi spots, the diameter of
the multi spots on the sample is made smaller, thereby
performing the irradiation with the multi-spot excita-
tion lights or the sheet-shaped excitation lights.

10   Here, the multi-spot excitation lights include a large
number of M microscopic spots the diameter of which is
smaller than 3 μm and larger than 0.3 μm, and the
sheet-shaped excitation lights have a focus-achieving
width that is smaller than 3 μm and larger than 0.3

15   μm. The fluorescent lights generated by the irradia-
tion from the respective multi spots or the respective
sheet-shaped irradiation locations are separated from
the excitation lights, then detecting the fluorescent
light images emitted from the sample with the use of a

20   plurality of weak light detecting components. Signals
obtained from the respective detecting components are
stored individually. Then, the relative positions
between the multi-spot excitation lights or the sheet-
shaped excitation lights and the sample are changed,

25   thus storing the above-described signals in sequence.
This allows the signals to be stored and collected over
a desired range on the sample. Then, the fluorescent
light picture is constructed from the collected data,

thereby making it possible to inspect the DNA. The use
of the method as described above enhances the resolu-
tions of the detected images. Utilizing the enhanced
resolutions makes it possible to detect a to-be-

5   inspected DNA in a cell as the fluorescent light images
and to execute the DNA inspection of the cell in the
following way: For example, employing, as a sample, a
desired target DNA to which a fluorescent light
material has been added, the fluorescent light

10 material-added target DNA is hybridized with the
corresponding DNA among 1-chain DNAs in the cell.

FIG. 79 is an embodiment-illustrating diagram
of the fluorescent light detection in the present
invention and, in particular, of the fluorescent light

15 detection that is effective in the DNA inspection. The
same reference numeral as that in FIG. 71 denotes the
same component. Instead of the multi-spot excitation
lights illustrated in FIG. 71 the irradiation of which
is performed with the spacing of 10 pixels, sheet-

20 shaped excitation lights 2000 are employed. As is the
case with the optical system in FIG. 70, fluorescent
lights obtained by the irradiation with the excitation
lights are separated from the excitation lights, then
being detected by the 1-dimensional photomultiplier

25 tube array or the 1-dimensional ultra-high sensitivity
censor. The excitations by the sheet-shaped excitation
beams are executed simultaneously over mutually
adjacent, for example, 50 pixels in a 1-dimensional

direction, then being detected simultaneously by the 1-dimensional high sensitivity censor. The scanning by the amount of, for example, 500 pixels is performed in the x direction perpendicular to the sheet-shaped beams

5 that is longer in the y direction, thereby obtaining fluorescent light images by the amount of 50×500 pixels. Next, the sheet-shaped beams are displaced by the amount of 50 pixels in the y direction, then executing the above-described one scanning. Repeating

10 this operation 10 times finally obtains the fluorescent light picture of 500×500 pixels. In the present embodiment, the background noise becomes slightly larger in comparison with the above-described embodiments where there is performed the irradiation with the

15 isolated spot light or the spot lights separated with each other with the spacing therebetween. Nevertheless, in comparison with the cases where the irradiation is performed largely in the 2-dimensional plane-like manner, it is possible to execute the fluorescent

20 light detection with a less noise. The signals obtained from the 1-dimensional photomultiplier tube array or the 1-dimensional ultra-high sensitivity censor are weak lights, and accordingly the photon counting is executed.

25   FIG. 80 illustrates an embodiment for indicating a method of generating the multi-spot excitation lights used for the fluorescent light detection or the DNA inspection using the fluorescent

light detection. In a laser 27g, there are provided
windows at Brewster angle at both ends of a laser tube
271 filled with a laser medium. Laser lights emitted
out therefrom are returned back by 100% reflection

5 mirrors 272, 273. The energy of the laser light that
is reciprocating between the resonator mirrors is
usually more than 10 times as high as the beam energy
extracted out of the laser resonator through the
emitting window from the optical energy within the

10 resonator. In the case of inserting a multi-spot or
sheet-shaped beam generating hologram plate 270 into
the laser resonator, since a hologram 2701 has been
formed in advance so that the multi spots or the sheet-
shaped beams are generated, as illustrated in the

15 drawing, multi spots 2702 are reproduced through a lens
274. Incidentally, the laser light with which the
hologram has been irradiated forms the multi-spot
images at a diffraction efficiency of substantially
10%. The remaining light passes through the hologram

20 without being varied as a 0th light, then reciprocating
within the laser resonator. These multi spots are
completely identical to the multi-spot lights at the
time of immediately after having passed through the
mask-like circular aperture array explained in FIG. 70.

25 This condition makes it possible to irradiate a detec-
tion target with the multi spots with the use of the
lens 24g in accordance with the configuration in FIG.
70. As a result, it is possible to obtain the multi-

spot lights or the sheet-shaped beams the intensities of which are almost 10 times higher as compared with those in the conventional case where the hologram is located in a laser-emitting optical path. Conse-

5 quently, it becomes possible to execute the high-speed and high-sensitivity fluorescent light detection and DNA inspection.

In the above-described embodiment, the explanation has been given concerning the case of the

10 excitation lights having 1 wavelength. The present invention, however, also exhibits the effects in the case of using the excitation lights having 2 wave-lengths or 3 or more wavelengths. In this case, conventionally, when trying to execute the detection

15 using the plurality of wavelengths, it has taken an exceedingly long time to do so. On the other hand, the execution of the photon counting detection using the multi-spot lights according to the present invention makes it possible to execute the detection with a high-

20 sensitivity and at a high-speed.